STUDY OF CHEMICAL CONSTITUENTS, PHARMACOLOGICAL ACTIVITIES ON THE WHOLE PLANT EXTRACTS OF *TRICHOSANTHES CUCUMERINA LINN*

Dissertation Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

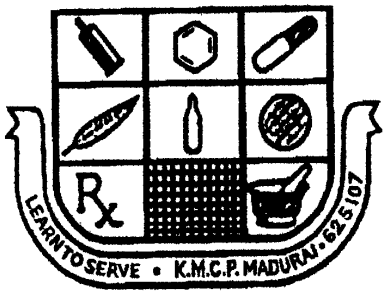
IN

PHARMACEUTICAL CHEMISTRY

of

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

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CERTIFICATE

This is to certify that the dissertation entitled **"STUDY OF CHEMICAL CONSTITUENTS, PHARMACOLOGICAL ACTIVITIES ON THE WHOLE PLANT EXTRACTS OF *TRICHOSANTHES CUCUMERINA LINN*"** submitted by **Mr. P.NATARAJ** to The Tamilnadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment of the requirement for the award of **Master of Pharmacy** in Pharmaceutical chemistry at K.M. College of Pharmacy, Madurai. It is a bonafide work carried out by him under my guidance and supervision during the academic year 2010-2011.

GUIDE
Mr.J.RAAMAMURTHY .M.PHARM;
Professor,
Dept. of Pharmaceutical chemistry,
K.M. College of pharmacy,
Uthangudi, Madurai-625107,
Tamilnadu.

H.O.D

Dr.S.Venkataraman, M.Pharm, Ph.D,
Professor & Head,
Dept. of Pharmaceutical chemistry,
K.M.College of Pharmacy,
Uthangudi, Madurai-625107,
Tamilnadu.

PRINCIPAL

Dr.S.Jayaprakash, M.Pharm, Ph.D.,
Professor & Head,
Dept. of Pharmaceutics,
K.M.College of Pharmacy,
Uthangudi, Madurai-625107,
Tamilnadu.

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NATARAJ.P

INTRODUCTION[1-12]

"*The emerging new technologies have significantly contributed in the advancements in developing new phytopharmaceuticals and food herbs, which are definitely going to alter the future outlook of family physicians and common people. India can play major role in the global market for herbals, herbal products, raw materials and isolated phytopharmaceuticals because of its extensive flora and fauna, expertise, trained technocrats and great plant heritage from Ayurveda and other resources*"

Nature was the main source of drug in ancient times, the progress and development of the human race is inseparably linked to the increasing scientific knowledge.

Green plants are essential for all animal life, since they convert solar energy into organic carbon compounds which is used as a basic energy for animals.

The curiosity of the present day man probes into the past and brings to light even fragmentary information about traditional methods of our ancestors, and it makes a fascinating study.

The world Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and native American Indian medicine. WHO notes that of 119 plant – derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducing extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

In Asia there are many traditional systems, they are Siddha and Ayurveda which are purely Indian systems. Unani system in Persia, Chinese traditional medicine, Japanese traditional medicine, Tibetian traditional medicine, and these systems are even now practiced.

In our country, the Ayurveda system of medicines was firmly believed to have originated from the Vedas and ancient religious scripts. In fact, there were strong convincing and asserting claims that Ayurveda was a divine gift and celestial benediction to the Indian people.

In short, the magic of herbs and plants are there all around us waiting to be discovered, understood and used. Because, they are now definitely recognized and accepted as perennial storehouses of infinite, limitless benefits to man.

The expectation was that Ayurvedha originated from the atharvanaveda and therefore was a divine dispensation. This magic spell even now is strongly vibrant in India, despite the advances and claims of scientific discoveries, dramatically brought about by logical explanation and exploration of the western systems.

Because of the limitation of western medicine, especially in the treatment of chronic disease e.g. asthma, hypertension, cancer, liver disorder and in the occurrence of adverse effect with certain synthetic drugs are traditional system has since about 1950, been undergoing a period of reassessment.

Herbal drugs are probably the most common source of samples for evaluation in high – throughout screens of natural products. They have yielded many useful compounds and plant-derived ingredients, which are important components of modern phytopharmaceuticals. Today, the global market is floated with herbal preparations. A number of companies, including some multinational are entering into the area of herbal medicines.

Constituent of the component drug and reasonable mechanism of action to explain the therapeutic abilities, the elucidation of which must be a goal of oriental medicine research, plant whose constituents are isolated are used in

allopathic medicine e.g. Atropine sulphate I.P.,Quinine sulphate I.P., Digoxin U.S.P.etc.

The disadvantage of oriental medicine is that the clinical use of drug is empirical and has been based on observation from clinical trials without experimental support. On the other hand as a great advantage, the efficacy has been already confirmed with humans. The development of science of phytopharmaceuticals in the western countries gave an impetus to the search for active principles in plants of Indian origin.

W.H.O GUIDELINES FOR ASSESSMENT OF HERBAL MEDICINES

Every herbal formulation must be standardized as per WHO guidelines. WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programmes, in assessing safety and efficacy and in ensuring adequate supplies and the quality control of raw and processed materials. HI According to WHO guidelines less stringent selection procedures could be applied for the screening, chemical analyses, clinical trials and regulatory measures but the procedure for pure phytochemicals for quality control should be identical to that for synthetic drugs according to WHO guidelines.

The World Health Organization (WHO) has recently defined traditional medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The traditional preparations comprise medicinal plants, minerals, organic matter, etc.

The manufacturing procedure and formula including the amount of excipients should be described in detail. The method of identification, and where possible quantification of the plant material in the finished product should be defined. If the identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g., chromatographic fingerprint) to ensure consistent quality of the product.

According to WHO, "Herbal Medicines" should be regarded as, "Finished, labeled medicinal products that contain active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substance of this nature. Herbal medicines may contain excipients in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, including, isolated constituents of plants are considered to be herbal medicines. Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients which are not of plant origin.

Multi-component botanical formulations can be standardized with newer techniques such as DNA fingerprinting, high pressure thin layer chromatography (HPTLC), liquid chromatography, and mass spectroscopy. The value of animal testing to establish safety and toxicity is not so critical if the botanicals are used in traditional forms. Nevertheless all the critical pharmacopoeial tests such as dissolution time, microbial, pesticide and heavy metals contamination etc. must be in accordance with global standards and all the Ayurvedic medicine manufacture must be in accordance with current good manufacturing procedures for herbs.

CURRENT STATUS AND THE FUTURE

The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. However, recent findings indicate that all herbal medicines may not be safe as severe consequences were reported for some herbal drugs. Most herbal products on the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Thousands of years traditional use can

provide us with valuable subjected to the selection, preparation and application of herbal formulation, to be accepted as viable alternative to modern medicine, the same vigorous method of scientific and clinical validation must be applied to prove the safety and effectiveness of a therapeutical product in the present review. We attempted to describe the present scenario and project the future of herbal medicine.

RECENT APPROACHES: RESEARCH AND DEVELOPMENT

There is a great demand for herbal medicines in the developed as well as developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lesser costs. Since herbal medicines are prepared from materials of plant origin they are prone to contamination deterioration and variation in composition. This gives rise to inferior quality of herbal products with little or no therapeutic efficacy.

HERBAL MEDICINE SCENARIO IN INDIA

The turnover of herbal medicines in India as over the counter products, ethical and classical formulations and have remedies of Ayurveda, Unani and Siddha systems of medicine is about $1 billion with a meager export of $ 80 million. 80% of the exports to developed countries are of crude drugs and not finished formulations leading to low revenue for the country. The list of medicinal plants exported from India are Aconitum species (root) Acorus *calamus* (rhizome), *Adatoda vasica* (whole plant), *Berberis aristata* (root), Cassia *augustifolia* (leaf and pod), *Colchicum luteum* (rhizome and seed), *Hedychium spicatum* (rhizome), *Heradeum candicans* (rhizome), *Inuia racemose* (rhizome), *Juglans reya* (husk), *Juniperus conimunis* (fruit), *Juniperus macropoda* (fruit), *Picrorhizn kurroon* '(root), Plantago ovata (seed and husk), *Podophyllum emodi* (rhizome), *Pinicn. yanatum* (flower, root and bark), *Rauwolfia serpentina* (root). Rheum *emodi* (rhizome), Saussurea (rhizome), *Swertia shirayita* (whole plant), *Valerian- intamansi* (rhizome), Zingiber *officinale* (rhizome) Five of these, i.e. Glycerrhiza glabra, *Commiphora mukut,* Plantago *ovata. Aloe barbadensis* and

Azardica indica are used in modern medicine. Others are used in 52 to 141 herbal formulations and Triphala (Terminalia *chebula,* and *Emblica officinalis]* along is used in 219 formulation.

India with its vast area from Kashmir to kanyakumari and varying soils and climatic conditions is a large store house of medicine plants, to be aptly called the "botanical garden of the world" and has a rich heritage of indigenous drugs from the Ayurvedic items.

The classical medicine system Ayurveda is strictly of Indian origin and development and it is still widely practised in India. More than 1500 remedial treatment with Indian medicine flora have been reported by Sushrata, Charaka and Vegbhatta in Sanskrit. The literature contain information about morphological features of many drugs, their geographical distribution and optimum condition for growth, the best season for their maximum potency as well as toxic properties thus a definite basis exists for investigating these plants for bioactive constituents.

The chemical analysis of crude drug helps us determining the action of medicine in health and disease. Today's emphasis of pharmaceutical research and development is on the search for the therapeutic substances with specific functions and minimum side effects in particular application. The plant derived substances having the advantages of being tools for medicine. Many different types of receptors were identified with the help of phytoconstituents e.g. muscarinic an active constituents of poisonous mushroom (amantia muscarrnia) was used to find both muscarinic & nicotinic receptors.

The natural plant product often serves as chemical models or templates for the design and total synthesis of new drug entities, e.g. new drug can be designed with the help of phytoconstituent, e.g. from morphine more than 25 synthetic congeners have been derived.

The wonder drugs of plant reported in recent years are ginsensosides from panx ginseng and have acquired commercial significance in view of its aphrodisiac and general tonic properties.

During the past decade investigation on secondary plants constituents have made phenomenal plant constituents have made phenomenal advances and thanks to the development of efficient separation techniques like column, thin layer, high pressure liquid and gas chromatography as well as sensitive methods of instrumental analysis such as UV, IR, NMR, ESR, ORD, CD and mass spectroscopy.

Modern instrumentation techniques have also made feasible, the study of micro quantities of substances with considerable precision in determining their chemical structure and distribution patterns in plants.

Recent reviews and book high light such investigation on the chemistry of medicinal plants will be more fruitful by close investigation with pharmacological and clinical investigation.

The technology involved in the extraction of pharmaceutical significance in majority of the cases is the guarded secret of the pharmaceutical or chemical firm. The method of extraction of phytopharmaceuticals represent the co-ordination of research work carried out by scientist different disciplines with the advancement in analytical and instrumentation technology, it has been possible to devise commercially feasible techniques for extraction of several phytochemicals.

The future of phytopharmaceuticals is bright as it undoubtedly serves as a cheep and steady for varied of therapeutic agents which are of great significance in the health care of mankind.

CONTENTS

REVIEW OF LITERATURE [13-41]

CHEMICAL CONSTITUENTS

- The chemical composition of cucurbitaceous plant belongs to aminoacids, fatty acids,sterol,triterpenes.The characteristic phytosterols occurring in this family are 24- Ethyl- sterols such as 24- Ethyl-cholest-7-en-ol,24-ethyl-cholesta-7,22-dieneol,24-ethyl choleta-7, 25-diene-ol, and 24-ethyl-choleta-7, 22,25-triene-ol,as the major sterol components and cucuribitacins are predominantly found in the cucurbitacin family, but are also present in several other families of the plant kingdom. The cucurbitacins may results in serious poisoning and even death.
- *Tricosanthes cucumerina* is a rich source of nutrition. It is highly constituted with proteins, fat, fibre, carbohydrates, vitamin A and E. The total phenolics and flavanoids content is 46.8% and 78.0% respectively. The fruit is rich in Vitamin C and E. The crude protein content is 30.18%. The predominant mineral elements were potassium (121.60mg,100:1g) and phosphorus (135.0mg,100:1g). Other elements found in fairly high amounts of Sodium, Magnesium and Zinc. Its reported by Yusuf AA et al.

- The triterpenes found are 23, 24-dihydrocucurbitacin D, 23,24-dihydrocucurbitacin B, cucurbitacin B, 3β-hydroxyolean-13(18)-en-28-oic acid, 3-oxo-olean-13(18)-en-30-oic acid and the sterol 3-*O*-*β*-D-glucopyranosyl-24-α-ethylcholest-7,22-dien-3*β*-ol. The percentage of free fatty acid and acid values were low suggesting increased stability and usefulness in nutritional and industrial applications. . Its reported by Frahm AW et al.

- The chemical constituents present in *T.cucumerina* are cucurbitacin B, cucurbitacin E, isocucurbitacin B, 23,24-dihydroisocucurbitacin B, 23,24-dihydrocucurbitacin E, sterols 2 *β*-sitosterol stigmasterol. Low amount of

chemical substances like oxalate, phytates and tannins were also present. Analysis showed that the seed of *T.cucumerina* have high oil content up to 42.5±5%. The presence of common protein bands among the species may be an evidence of evolutionary origin and many protein bands found to be unique in the *Trichosanthes cucumerina* suggested that there is no genetic relationship with *Lycopersicon*. . Its reported by Ekam VS et al.

- A galactose-specific lectin and ribosome-inactivating protein named trichoanguin are present in aerial parts. The bulk of carotenoids made of lutein is present in the concentration of 15.6 -18.4 mg/100ml FW15. Circular dichorism spectroscopic studies reveal that TCSL contains about 28.4% beta-sheet, 10.6% beta-turns, 7% polyproline type 2 structure, with the remainder comprising unordered structure; the alpha-helix content is negligible. The α- carotene contents were 10.3 - 10.7 mg/100ml FW and the β- carotene contents were found to be 2.4 - 2.8 mg/100ml. The ascorbic acid content found was 24.8 – 25.7 mg/100g fresh weight and lycopene content was16.0 and 18.1 mg/100g . . Its reported by Bhide SV, Chow LP et al.

- Chemical modifications carried out with imidazole side chains of histidine residues with ethoxyformic anhydride on the galactose-specific lectin (SGSL) purified from snake gourd. Trichosanthes seeds indicated that the loss of activity upon modification was not due to changes in the overall conformation of the lecithin. A novel isoflavone glucoside, 5,6,6'-trimethoxy-3',4'-methylenedioxyisoflavone7-O-beta-D-(2''-O-p-coumaroyl glucopyranoside) has been characterized from the seeds of *Trichosanthes*. The positive effects of the plant are due to the carotenoids, flavanoids, lycopene, phenolics and ß-carotene present in it. Its reported by Swamy MJ,Padma P et al.

- **Antiproliferative Effect** of Cucurbitacin B extracted from *Trichosanthes cucumerina* L. on Human Cancer Cell Lines reported by Tanawan Kummalue, M.D., Weena Jiratchariyakul, Dr.rer.nat., Totsaporn Srisapoomi, M.S., Sathien Sukpanichnant, M.D., Toshiro Hara, M.D., Ph.D., Kenzaburo Tani, M.D., Ph.D et al.

- Physiochemical and saccharide-binding studies on the galactose-specific seed lectin from Trichosanthes cucumerina reported by Kenoth R, Komath SS, Swamy MJ.et.al. (Physiochemical and saccharide-binding studies have been performed on Trichosanthes cucumerina seed lect). The agglutination activity of TCSL is highest in the pH range 8.0-11.0, whereas below pH 7.0 it decreases quite rapidly, which is consistent with the involvement of imidazole side chains. The lectin activity is unaffected between 0 and 60 degrees C, but a sharp decline occurs at higher temperatures Thermodynamic and kinetic analysis of porphyrin binding to Trichosanthes cucumerina seed lectin. Kenoth R, Raghunath Reddy D, Maiya BG, Swamy MJ.et.al. The interaction of several metallo-porphyrins with the galactose-specific lectin from Trichosanthes cucumerina (TCSL) has been investigated. Difference absorption spectroscopy revealed that significant changes occur in the Soret band region of the porphyrins upon binding to TCSL and these changes have been monitored to obtain association constants (Ka) and stoichiometry of binding.

- Steady-state and time-resolved fluorescence studies on Trichosanthes cucumerina seed lectin reported by KenothR. Swamy MJ et.al.

- Comparative evaluation of **hypoglycaemic activity** of Trichosanthes cucumerina in alloxan diabetic rats reported by Kar A, Choudhary BK, Bandyopadhyay NG. et.al. In our experiments 30 hypoglycaemic medicinal plants (known and less known) have been selected for thorough studies from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines. In all the experiments with different herbal samples

(vacuum dried 95% ethanolic extracts), definite blood glucose lowering effect within 2 weeks have been confirmed in alloxan diabetic albino rats. Blood glucose values are brought down close to normal fasting level using herbal samples at a dose of 250 mg/kg once, twice or thrice daily, as needed.

- **Antiproliferative Effects** of Cucurbitacin B in Breast Cancer Cells: Down-Regulation of the c-Myc/hTERT/Telomerase Pathway and Obstruction of the Cell Cycle in Trichosanthes cucumeirna reported by Duangmano S, Dakeng S, JiratchariyakulW, Suksamrarn A, Smith DR, Patmasiriwat P et al. Naturally occurring cucurbitacins have been shown to have anticancer, antimicrobial and anti-inflammatory activities. In this study, we determined the effects of cucurbitacin B extracted from the Thai herb Trichosanthes cucumerina L. on telomerase regulation in three human breast cancer cell lines (T47D, SKBR-3, and MCF-7) and a mammary epithelium cell line (HBL-100). Cell viability after treatment with cucurbitacin B, which is an active ingredient of this herb, was assessed. Telomeric repeat Amplification Protocol (TRAP) assays and RT-PCR (qualitative and realtime) were performed to investigate activity of telomerase as well as expression of human telomerase reverse transcriptase (hTERT) and c-Myc. The c-Myc protein level was also determined in SKBR-3 and HBL-100 cells. Our results show that the cucurbitacin B inhibits growth and telomerase activity in the three breast cancer cell lines and exerts an obvious inhibitory effect in the oestrogen receptor (ER)-negative breast cancer SKBR-3 cells.

- **Anti-inflammatory activity** of Trichosanthes cucumerina Linn.in rats. reported by Arawwawala M, Thabrew I, Arambewela L, Handunnetti S et.al. Apart from the lowest dose of the HWE, other tested doses (500, 750, 1000 mg/kg) produced a significant ($P \leq 0.05$) inhibition of the inflammation, most pronounced at 5h after the injection of carrageenan. The anti-inflammatory effect induced by 750 mg/kg, was comparable to that of the reference drug, indomethacin at 4 and 5h. Inhibition of nitric oxide (NO) production and membrane stabilization activities are probable mechanisms by which Trichosanthes cucumerina mediates its anti-

inflammatory actions. Among the tested fractions, methanol fraction (MEF) and aqueous fraction (AQF) at a dose of 75 mg/kg exhibited marked inhibition against carrageenan-induced hind paw oedema. The anti-inflammatory effect induced by MEF was comparable to that of the reference drug indomethacin and as well as to the 750 mg/kg of HWE at 4 and 5h.

- **Gastroprotective activity** of Trichosanthes cucumerina in rats reported by Arawwawala LD, Thabrew MI, Arambewela LS**.**

- Phyto-Constituents and **Anti-Oxidant Activity** of the Pulp of Snake Tomato *Trichosanthes Cucumerina* L. reported by O.C.Adebooye et.al. The phyto-constituents and antioxidant activity of the fruit pulp of *Trichosanthes cucumerina* L. have not been reported in literature and were therefore studied. Two identified morphotypes of this plant (Morphotype I [V1] having long fruit with deep green background and white stripes and Morphotype II [V2] having light green coloured long fruit were used for the studies. The dry matter contents of the pulp of the V1 and V2 were 10.9 and 9.6 g/100g fresh weight (FW), while the ascorbic acid contents were 25.7 and 24.8 mg/100g fresh weight (FW), and lycopene contents were18.0 and 16.1 mg/100g FW, respectively. The total phenolics, total flavanoids and total ferric reducing antioxidant power (FRAP) of V2 were significantly higher ($P< 0.05$) than that of V1 by 46.8%, 78.0% and 26.2%, respectively. Bulk of the carotenoids is made up of lutein in the concentration of 15.6 and 18.4 mg/100g FW, for V1 and V2, respectively. The α-carotene contents were 10.3 and 10.7 mg/100g FW while the β-carotene contents were 2.4 and 2.8 mg/100g FW for V1 andV2, respectively. It is concluded from the results of this study that the two morphotypes of *T. cucumerina* possess valuable nutraceutical properties that can qualify them as viable substitute to the Solanaceous tomato
- **Antiproliferative Effect** of Cucurbitacin B Extracted from *Trichosanthes cucumerina* L. on Human Cancer Cell Lines reported by Tanawan Kummalue, M.D, Weena Jiratchariyakul, Dr.rer.nat., Totsaporn

Srisapoomi, M.S., Sathien Sukpanichnant, M.D., Toshiro Hara, M.D., Ph.D., Kenzaburo Tani, M.D., Ph.D.

Anti-inflammatory activity:

- Kolte RM, *et al* in 1997 with hot aqueous extract of root tubers of *Trichosanthes cucumerina* have investigated against carrageenin induced mouse's hind paw oedema and it exhibited significant anti-inflammatory activity

Cytotoxic activity:

- Kongtun S et al in 1999 with the root extract of Trichosanthes cucumerina L and four human breast cancer cell lines and lung cancer cell line. The root extract inhibited more strongly than the fruit juice.

Hypoglycaemic activity

- Kar.A *et al* in 2003 with crude ethanolic extract of *Tricosanthes cucumerina L* showed significant blood glucose lowering activity in alloxan diabetic albino rats.

Larvicidal efficacy

- Rahuman.A.A *et al* in 2008 using the acetone extract of leaves of Tricosanthes cucumerina L showed moderate larvicidal effects

Anti-diabetic activity

- M Arawwawala, *et al* in 2009 using hot water extract of aerial parts of *Trichosanthes cucurmerina L* has noted to improve glucose tolerance and tissue glycogen in non insulin dependent diabetes mellitus induced rats. Study showed the drug possess antidiabetic activity with improvement in oral glucose tolerance and glucose uptake in peripheral tissues.

Hepatoprotective activity

- Sathesh Kumar.S, *et al* in 2009 found that the methanolic extract of the whole plant of Trichosanthes cucumerina showed good hepatoprotective activity against carbon tetrachloride induced heapatotoxicity.

Anti-fertility activity

- Devendra N. Kage, *et al* in 2009 showed the antiovulatory activity of ethanolic extract of whole plant of *Trichosanthes cucumerina* L. var. *cucumerina* in female albino rats.

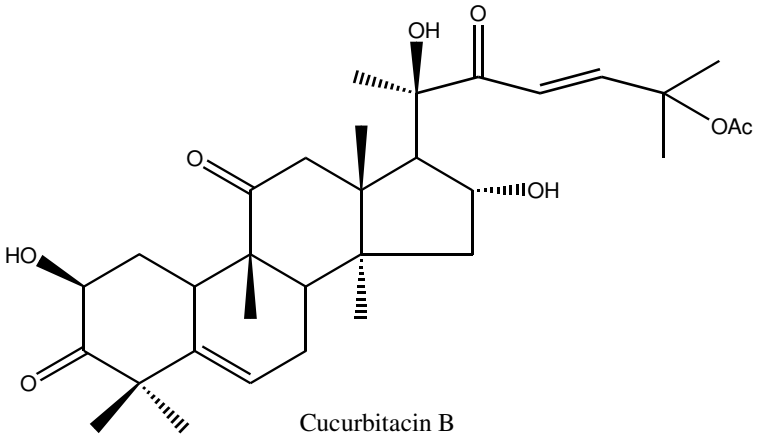
Gastroprotective activity

- Arawwawala LD *et al* in 2009 with hot water extract of Trichosanthes cucumerina L, showed a significant protection against ethanol or indomethacin induced gastric damage increasing the protective mucus layer, decreasing the acidity of the gastric juice and antihistamine activity. Dose dependent gastroprotective effects were observed in the alcohol model in terms of the length and number of gastric lesions mediated by alcohol in wistar stain rats.

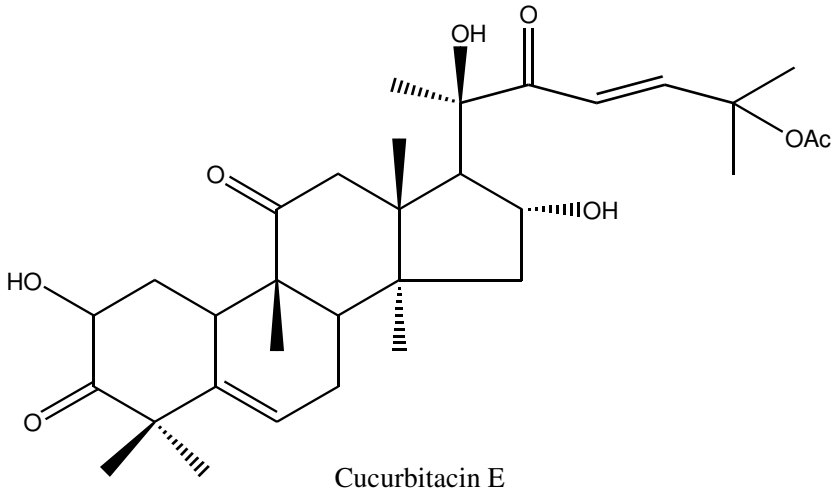
- Phyto-Constituents and Anti-Oxidant Activity of the Pulp of Snake Tomato (*Trichosanthes Cucumerina* L. reported by O C Adebooye et al. The phyto-constituents and antioxidant activity of the fruit pulp of *Trichosanthes cucumerina* L. have not been reported in literature and were therefore studied. Two identified morphotypes of this plant Morphotype I [V1] having long fruit with deep green background and white stripes; and Morphotype II [V2] having light green coloured long fruit) were used for the studies. The dry matter contents of the pulp of the V1 and V2 were 10.9 and 9.6 g/100g fresh weight (FW), while the ascorbic acid contents were 25.7 and 24.8 mg/100g fresh weight (FW), and lycopene contents were18.0 and 16.1 mg/100g FW, respectively. The total phenolics, total

flavanoids and total ferric reducing antioxidant power (FRAP) of V2 were significantly higher ($P< 0.05$) than that of V1 by 46.8%, 78.0% and 26.2%, respectively. Bulk of the carotenoids is made up of lutein in the concentration of 15.6 and 18.4 mg/100g FW, for V1 and V2, respectively. The α-carotene contents were 10.3 and 10.7 mg/100g FW while the β-carotene contents were 2.4 and 2.8 mg/100g FW for V1 andV2, respectively. It is concluded from the results of this study that the two morphotypes of *Trichosanthes cucumerina* L.

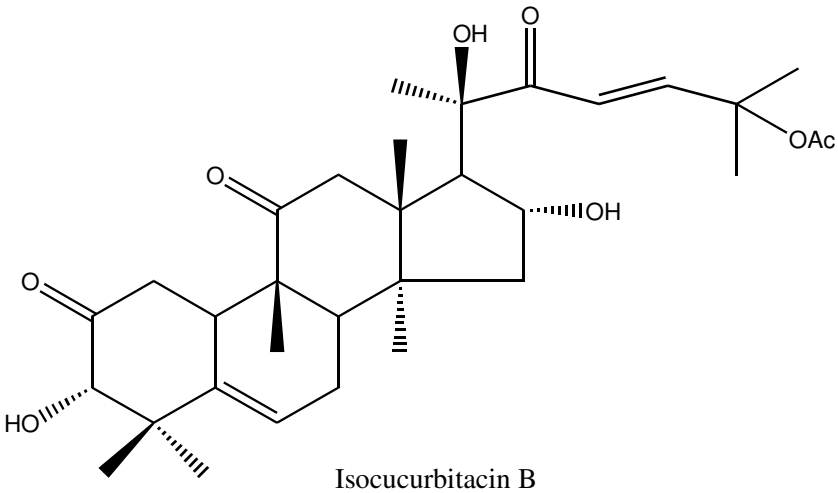
STRUCTURE :



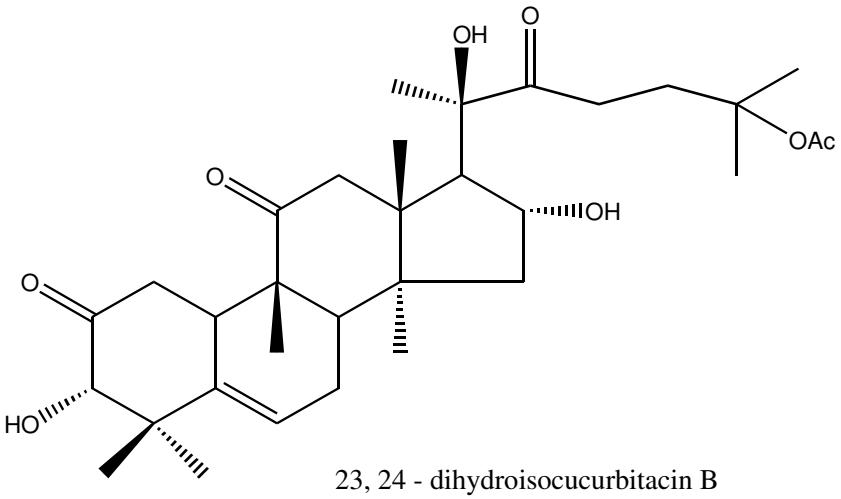
Cucurbitacin B



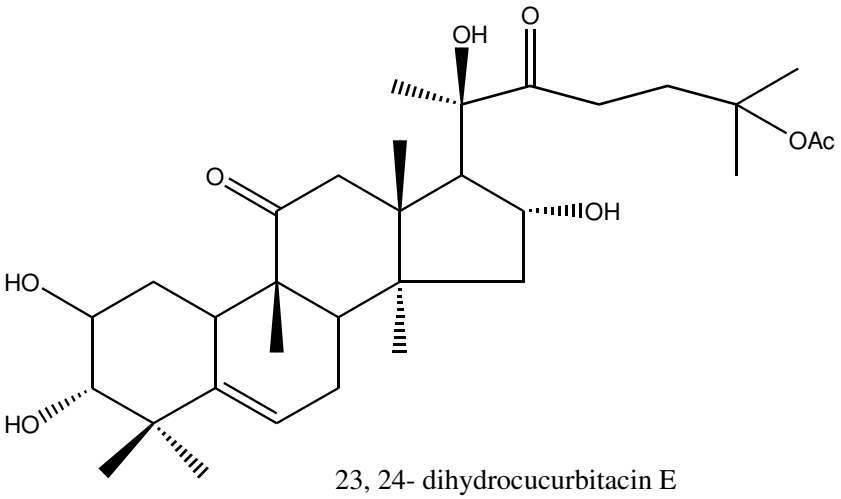
Cucurbitacin E



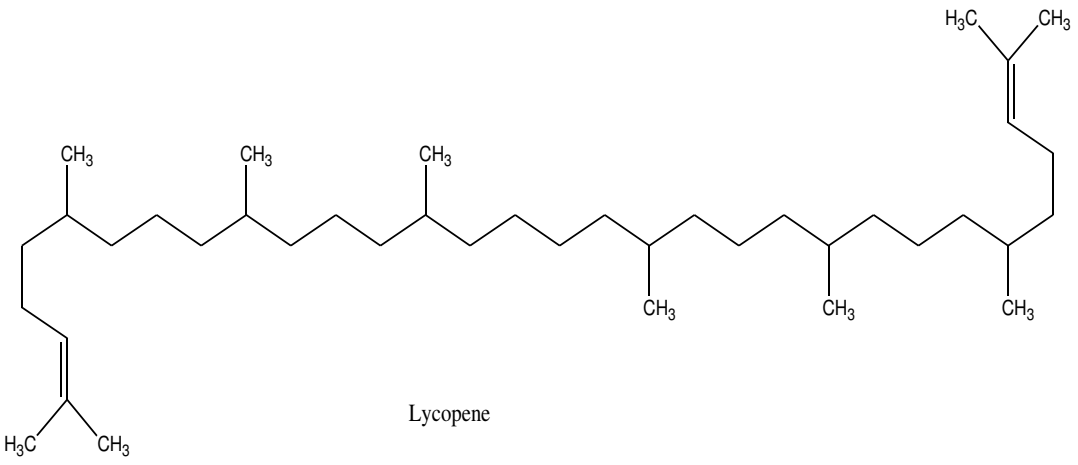
Isocucurbitacin B



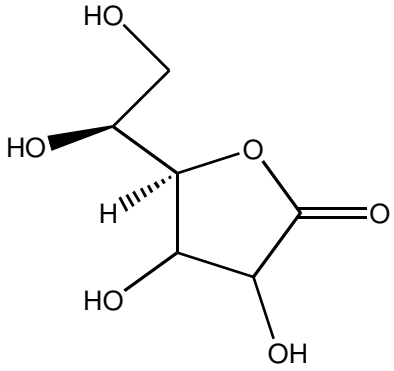
23, 24 - dihydroisocucurbitacin B



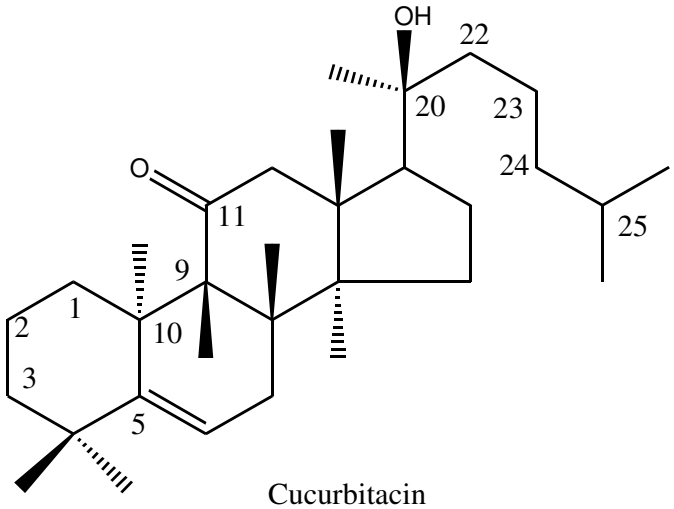
23, 24- dihydrocucurbitacin E



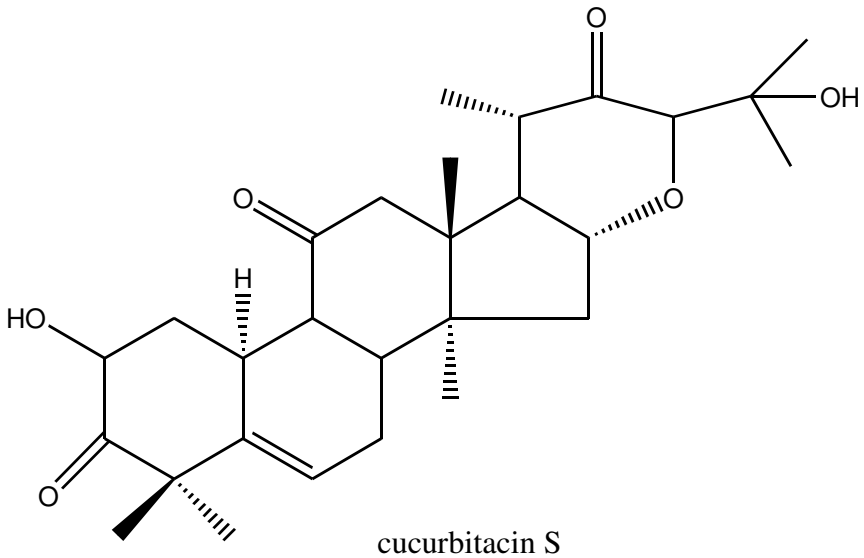
Lycopene



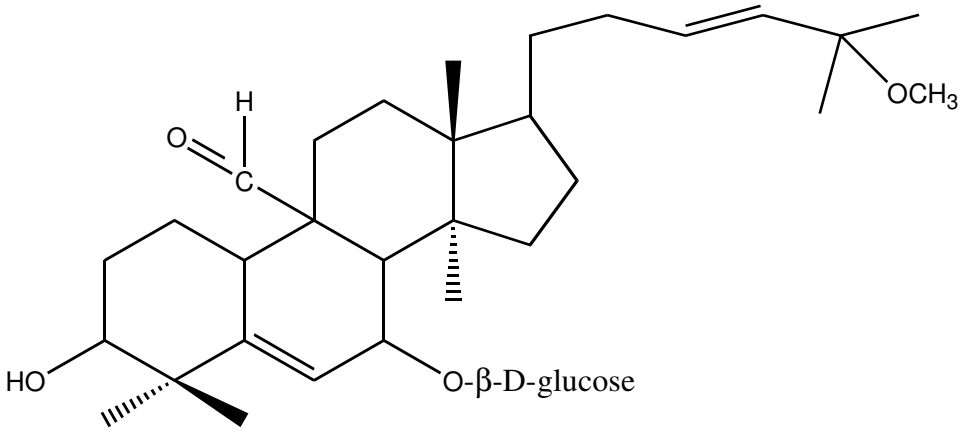
Ascorbic acid



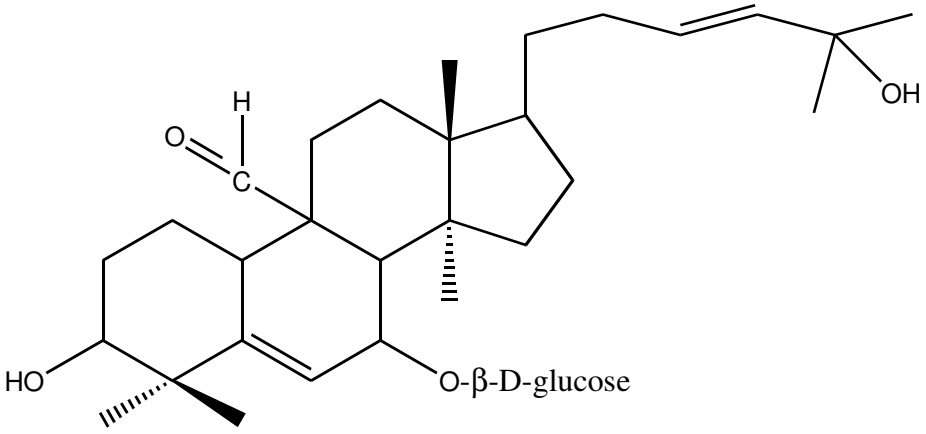
Cucurbitacin



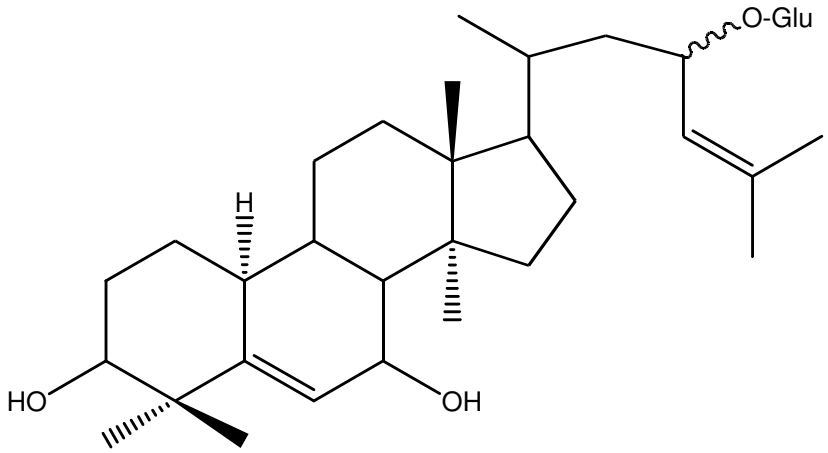
cucurbitacin S



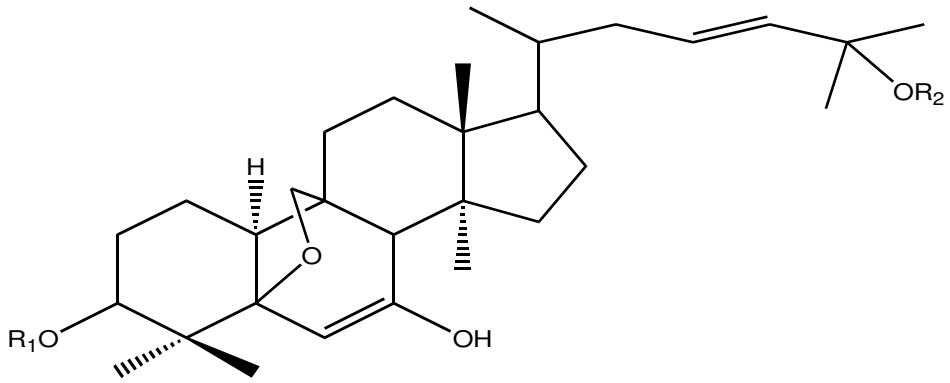
Momordicoside K



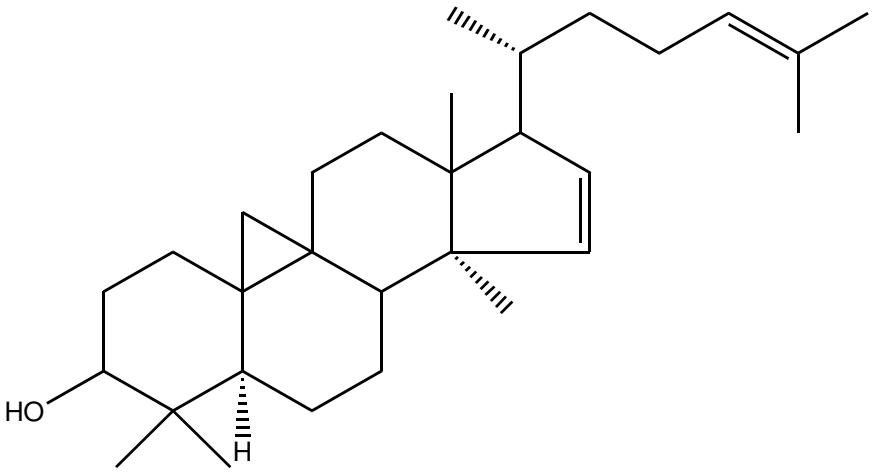
Momordicoside L



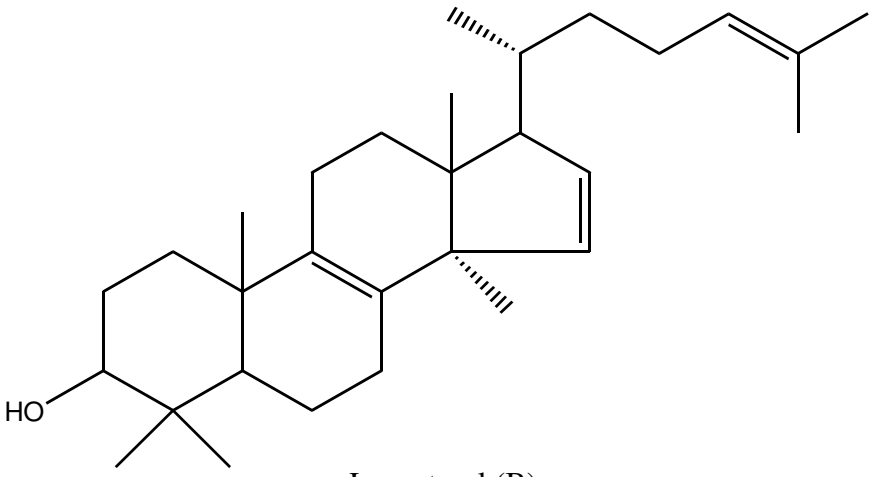
Momordicoside G : R_1 = β–D-allose; R_2 = CH_3
Momordicoside F_1 : R_1 = β–D-glucose; R_2 = CH_3
Momordicoside F_2 : R_1 = β–D-allose; R_2 = H
Momordicoside G : R_1 = β–D-glucose; R_2 = H



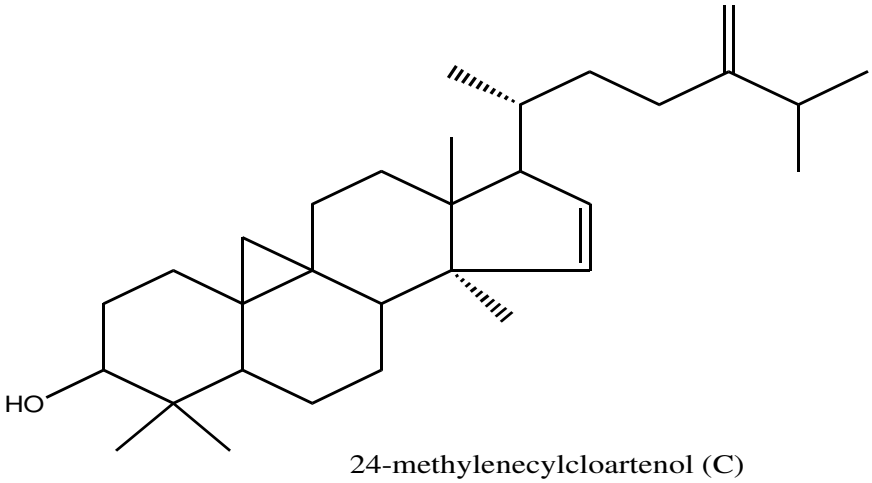
Momordicins I (R = H) and II (R = β–D-glucose)



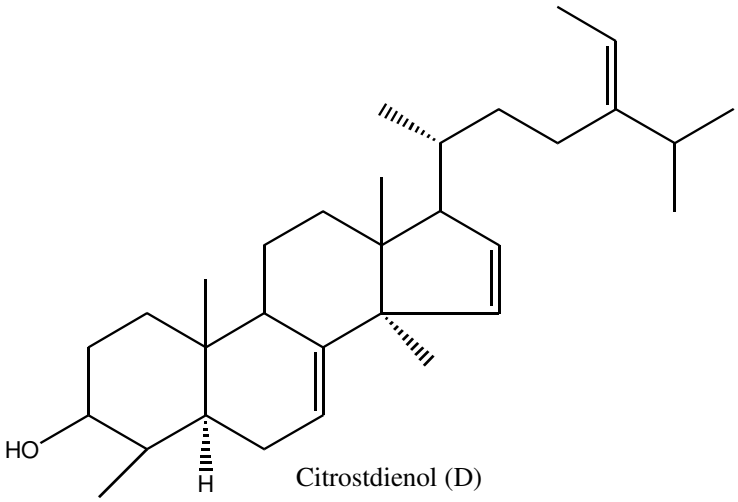
Cycloartenol(A)



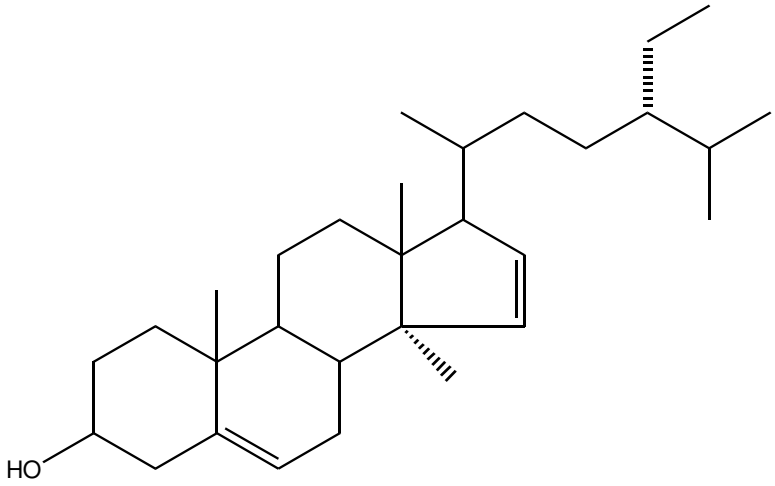
Lanosterol (B)



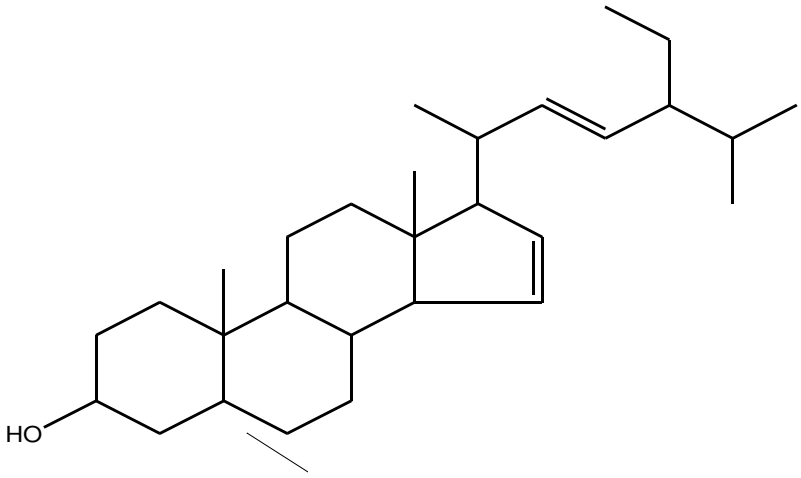
24-methylenecylcloartenol (C)



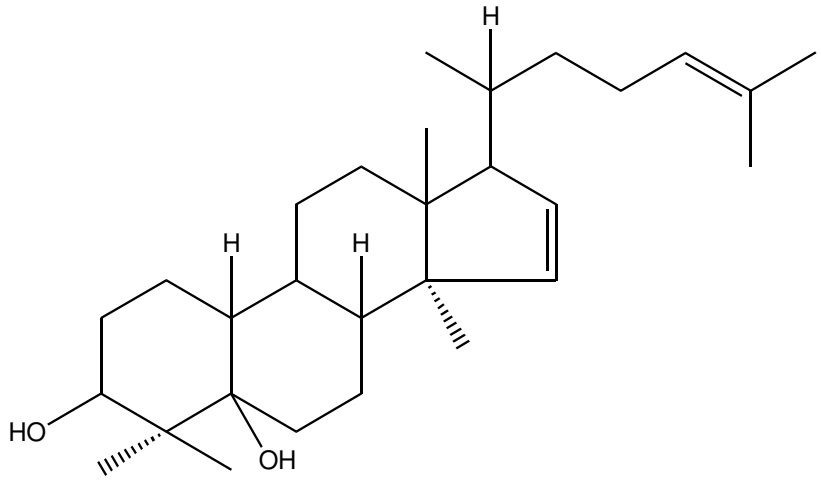
Citrostdienol (D)



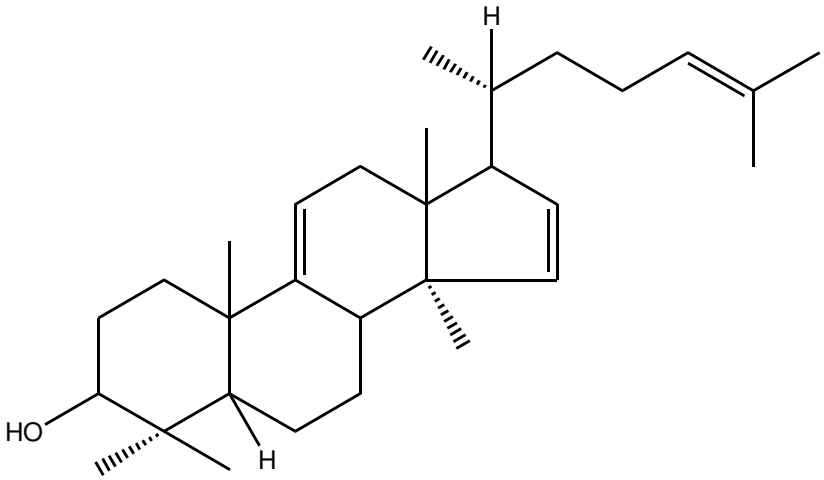
β-sitosterol (E)



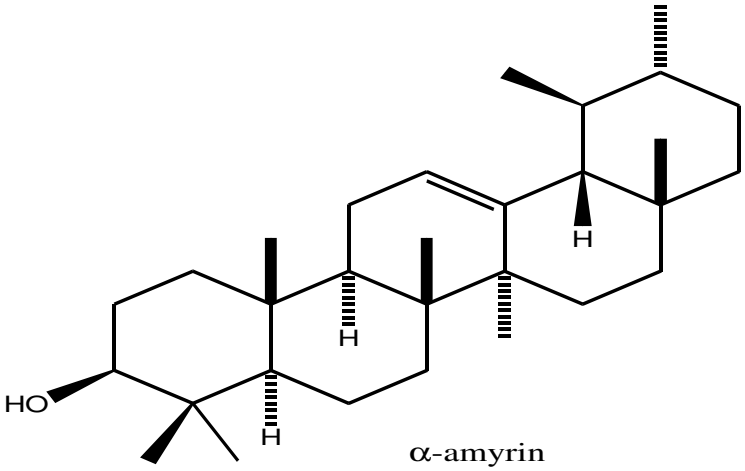
Stigmasterol (F)



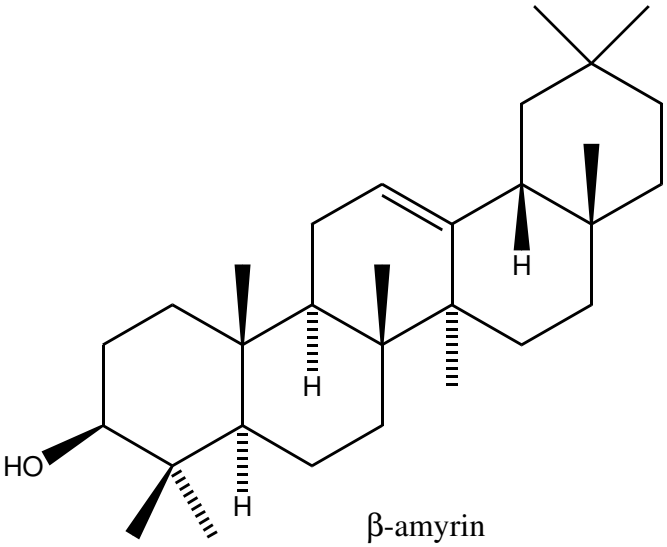
Litsomentol (G)



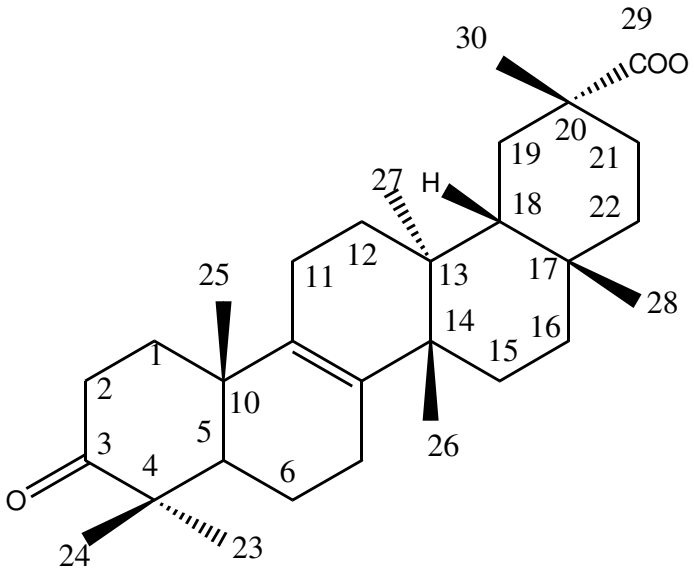
Parkeol (H)



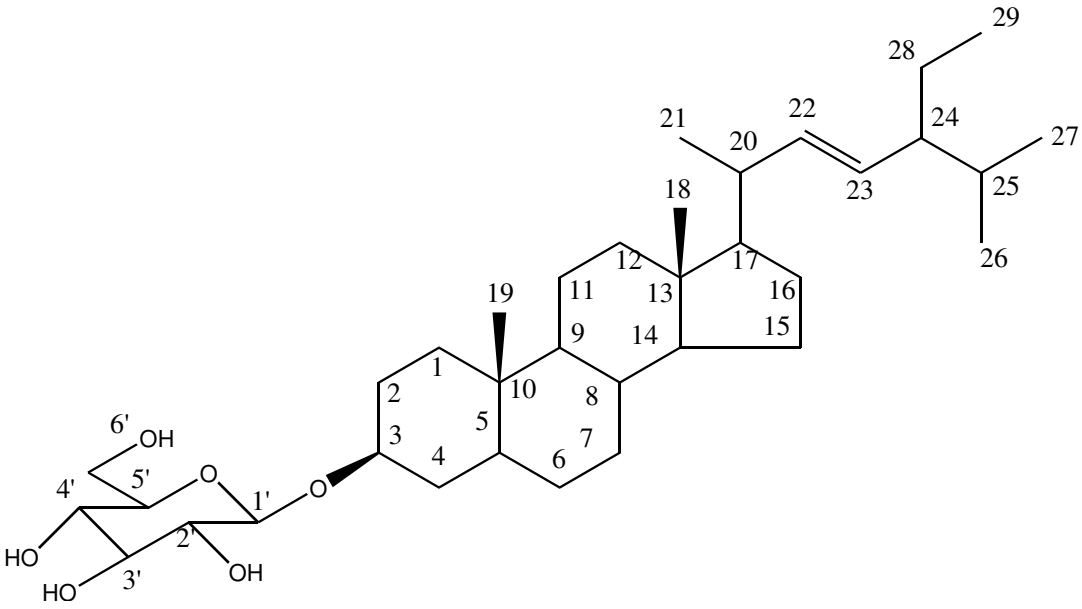
α-amyrin



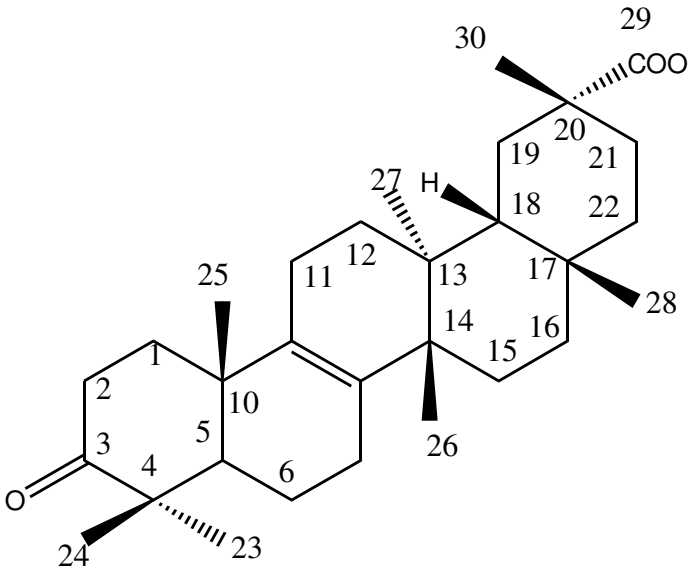
β-amyrin



BRYONOLIC ACID



CHONDRILLASTERYL GLUCOSIDE



BRYONONIC ACID

Morphology of the plant *Tricosanthes cucumerina Linn.*

PLANT DESCRIPTION[46-54]

BOTANICAL NAME : ***Tricosanthes cucumerina Linn.***

VERNACULAR NAMES:

Bengali	:	Chichinga (or) Chichinge
Sambalpuri	:	Purla
Telugu	:	Puttakoaya
Tamil	:	Pudalankai, peipudal
Assamese	:	Dhunduli
Kannada	:	Puduvalakaayi
Malayalam	:	Padavalanga
English	:	Snake gourd
Hindi	:	Cuccinda, paraval

SCIENTIFIC CLASSIFICATION:

Kingdom	:	Plantae
Division	:	Magnoliaphyta
Class	:	Magnoliopsida
Order	:	Curcubitales
Family	:	Curcubitaceae
Genus	:	Trichosanthes
Species	:	Cucumerina

The regional names of snake gourd or snake tomato is called in Bengali as Chichinga/ Chichinge, in Telugu as potlakaaya, in Tamil as pudalankaai, in Kannada as aduvalakaayi, in Malayalam as padavalanga, Galartori in Punjabi, padavali in Gujarathi, Chachinda in Hindi.

In other nations it is commonly called as serpent vegétal in France, Schlangengurke in Germany, Karasu-uri-zoku in Japan, Patola in Srilanka, Zucchetta cinese in Italy, Abóbora-serpente in Portugal, Käärmekurkku in Finland, Buap nguu Ma noi in Thailand, Yilan kabagi in Turkey, Calabaza anguina in Spain.

Non Insulin Dependent Diabetes Mellitus (NIDDM) also called as type 2 diabetes is a complex metabolic disorder that involves abnormalities in both insulin secretion and action at peripheral tissues. It is a more prevalent form of diabetes and responsible for 90% of the disease. In NIDDM, the kinetics of insulin release in response to meal or glucose is altered. So, postprandial blood glucose remains high and leads to glucose intolerance. Postprandial hyperglycemia plays an important role in the development of diabetic complications.[2] Poor glycogen content in insulin dependent tissues such as liver, skeletal muscle and adipose tissues were observed in NIDDM due to insulin resistance. *Trichosanthes cucumerina* Linn.

Belonging to family Cucurbitaceae is an annual climber and widely distributed in southern parts of India. Traditionally, decoction of the stem, leaves and aerial parts were used in the treatment of diabetes and inflammatory diseases. [4] The major active constituents of the drug are triterpenoid saponins viz., cucurbitacins. On the above evidence, the present investigation was planned to study the effect of aqueous extract of *Trichosanthes cucumerina* Linn. on NIDDM induced rats.

A complete understanding of medicinal plants involves a number of factors like botany, chemistry, genetics, quality control and pharmacology. In addition there is a large wealth of knowledge in the medicinal and other properties of plants from generation to generation by the tribal societies1. *Tricosanthes cucumerina* is a well known plant, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato.

The fruit is usually consumed as a vegetable due to its good nutritional value. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti inflammatory, larvicidal effects.

Various species :

- ✓ T. anguina
- ✓ T. baviensis Gagnepain
- ✓ T. cucumerina
- ✓ T. cucumeroides (Ser.) Maxim.
- ✓ T. dioica (Bengali: potol, green vegetable)
- ✓ T. dunniana Levl.
- ✓ T. fissibracteata C.Y. Wu ex C.Y. Cheng & Yueh
- ✓ T. homophylla Hayata
- ✓ T. kerrii Craib

- ✓ T. laceribractea Hayata
- ✓ T. lepiniana (Nuad.) Cogn.
- ✓ T. ovigera Blume
- ✓ T. pedata Merr. & Chun
- ✓ T. quinquangulata A. Gray
- ✓ T. rubiflos Thorel ex Cayla
- ✓ T. rugatisemina C.Y. Cheng et Yueh
- ✓ T. sericeifolia C.Y. Cheng et Yueh
- ✓ T. subrosea C.Y. Cheng et Yueh
- ✓ T. subvelutina F.Muell. ex Cogn.
- ✓ T. tricuspidata Lour.
- ✓ T. truncata C.B. Clarke
- ✓ T. villosa Blume
- ✓ T. wallichiana (Ser.) Wight

MORPHOLOGY CHARACTERS:

- ❖ Trichosanthes cucumerina is a monococus annual herb.
- ❖ Climbing by 2-3 branched tendrils upto 5-6 meters high or less.
- ❖ The stems are slender
- ❖ Green in colour
- ❖ 4 angled

Odour:

- ❖ Faintly disagreeable

Roots:

- Tuberous and whitish

Leaves:

- Alternate, simple with no stipules
- Scabrid hairy on both surfaces
- Rounded in outline
- 7 to 14 cm long and broad
- 3 or 5 lobed
- The lobes being broad, rounded or obtuse
- Sinuses broad (or) narrow and rounded

Base:

- It is broadly heart shaped

Staminate in florescence:

- These are long – peduncled and axillary
- 6-15 flowers

Flowers:

- Unisexual
- Regular
- White in colour with green and hairy calyx
- Corolla is tubular with lobes fringed and hair like growths
- The male flowers are many flowered with axillary racemes on 10 -30 cm long peduncles. They are with 3 stamens.
- Female flowers are solitary and sessile with inferior, single celled ovary, long and with hairy stigmas.

Fruits:

- Fruits are very slender
- Long and cylindrical berry
- Often twisted
- Greenish white when immature
- Dark red when mature

Seeds:

- These are half ellipsoid
- Compressed
- Undulate
- Hard
- Ragose
- Nearly one cm long
- Grayish brown in colour
- Sculptured
- Margin is undulate and imbedded

Origin and Distribution:

- Throughout India
- Trichosanthes is native to southern and eastern Asia
- Australia and Islands of the western pacific.
- It is grown as a minor vegetable in many countries of tropical Asia.
- It is locally grown as a vegetable in home gardens in Africa.
- Commercial growers around big cities in East.
- It is distributed in temperate Asian regions like China
- Tropical regions like Bangladesh, India, Nepal, Pakistan, Srilanka, Myanmar, Vietnam, Indonesia, Malaysia, Philippines. In Australia it is found in Northern Territory.
- Queensland and in Western Australia.

AIM OF THE PRESENT STUDY

In view of the Pharmacological, biological properties and chemical constituents of plant from Trichosanthes species, it was decided to study the whole plant of Trichosanthes cucumerina Linn, which is widely used in folk medicine.

The aim of this dissertation work was divided in to the following region.

- Preliminary Screening of crude extracts obtained after solvent extraction and partial purification by chromatography and chemical test analysis.
- Isolation and purification of selective phyconstituents.
- Characterization of purified compound by physical, chemical and spectal data.
- To study the pharmacological activity.

PHYTOCHEMICAL INVESTIGATION OF THE Trichosanthes Cucumerina linn [51-64]

Collection of plant materials:

The details regarding the description of plant were already given. The plants of Trichosanthes Cucumerina linn were collected from Madurai during the months of october and identified by Dr.Stephen Lecturer,American college,Madurai. The plants were then washed with water to remove soil and other extraneous matter. The wholeplant were cut into small pieces and were dried under shade for 20 days. Then the dried plant was homogenized to coarse powder and stored in airtight container.

Apparatus used for extraction and isolation of compounds:

Round bottom flask, bulb condenser, adaptor, column, TLC plates, test tube, conical flask, measuring cylinder, beaker, funnel, watch glass, thermometer, capillary tube.

Chemicals and solvents:

- Petroleum ether AR,
- Benzene AR,
- Chloroform AR,
- Ethyl acetate AR,
- Methanol AR,Silica gel.

EXTRACTS

- *Petroleum ether Extract*
- *Chloroform Extract*
- *Methanolic Extract*

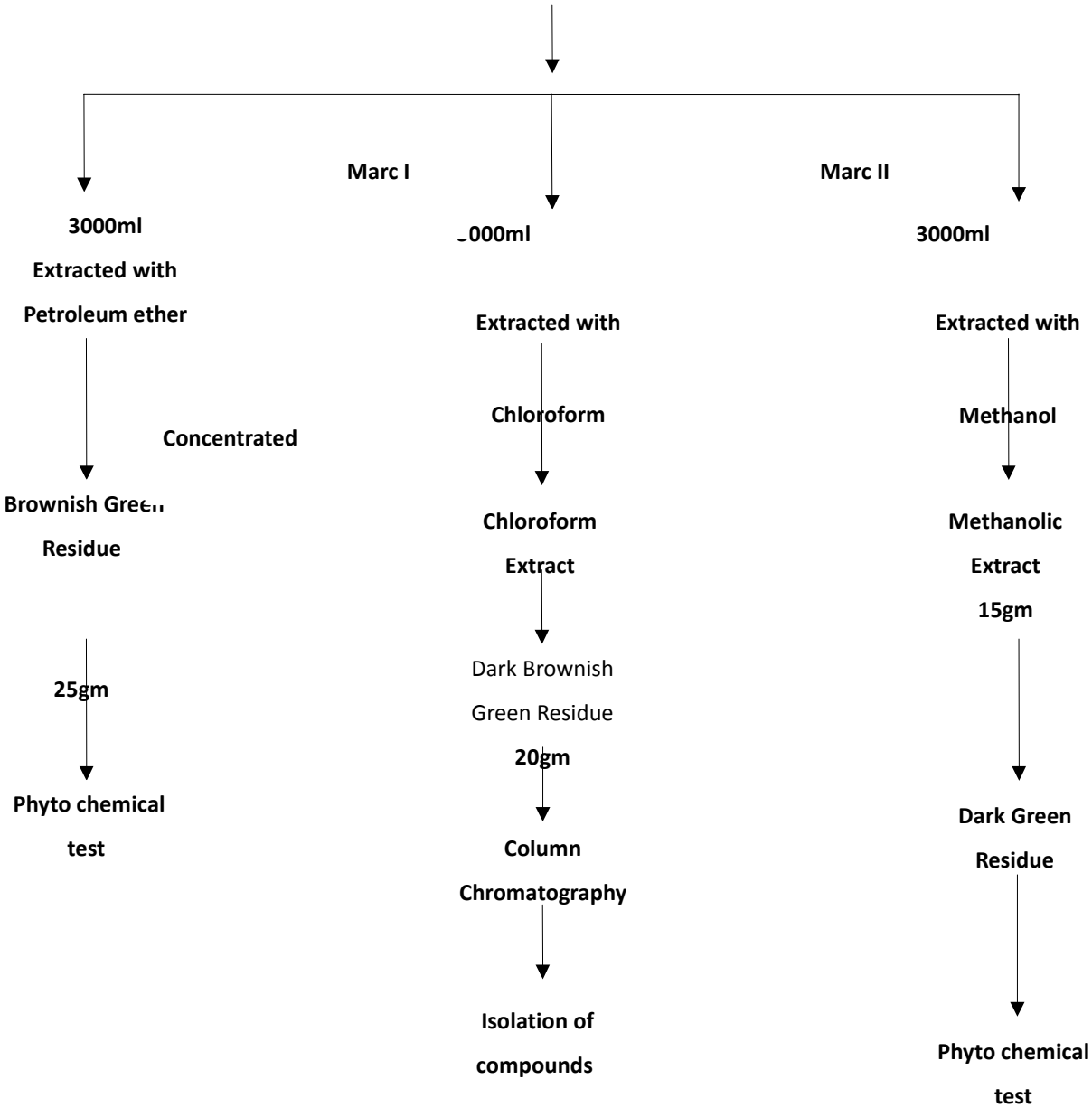
Method of Extraction:

About 500gms of dried coarse powder was soaked with petroleum ether (3000ml) for two days. After this, materials were extracted with petroleum ether (40°C – 60°C) by continuous hot percolation method for 72 hrs. The petroleum ether extract were filtered and concentrated under reduced pressure. A green-black residue was obtained (25gms). The marc left after the petroleum ether extraction were dried and extracted with chloroform (3000ml) for 72hrs. The chloroform extract were also filtered and concentrated under reduced pressure. A dark black residue was obtained (20gms).Then marc left after the chloroform extraction were dried and extracted with methanol (3000ml) for 72hrs. The methanolic extract were also filtered and concentrated under reduced pressure. A darkgreen residue was obtained (15gms).

FLOWCHART FOR VARIOUS EXTRACTION AND ISOLATION OF COMPOUNDS FROM *Trichosanthes cucumerina Linn*

SCHEMATIC DIAGRAM – I

Dry coarse material 500gm extracted by Hot continuous percolation Method using SoxhletApparatus



PRELIMINARY QUALITATIVE CHEMICAL EVALUATION

The extracts obtained by Trichosanthes Cucumerina linn was subjected to qualititative test for identification of various plant constituents.

1. DETECTION OF CARBOHYDRATE

★ Dissolved minimum amount of the extracts in 5ml of chloroform and filtered it. The filtrate was subjected to Molisch's test to detect the presence of carbohydrate.

2. Molish's test

★ Filtrate was treated with 2-3drops of 1% alcoholic α – naphthol and 2ml of concentrated sulphuric acid was added along the sides of test tube. Violet coloured ring was formed at the junction of the two liquids from the methanolic and chloroform extracts. Its showed the presents of carbohydrates.

3. DETECTION OF GLYCOSIDES

★ Small quantity of all extracts were hydrolyzed with hydrochloric acid for two hours in a water bath and the hydrosylate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

4. Legal's test

★ To the hydrosylate extract, 1ml of pyridine and few drops of sodium nitroprusside solution were addedand then it was made alkaline with sodium hydroxide.

★ Pink to yellow colour was obtained in allthree extracts showed the presence of glycosides.

5. Borntrager's test

★ The extracts were treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added.

★ Appearance of pink colour was observed inallextracts, which showed the presence of glycosides.

6. DETECTION OF PHYTOSTEROLS

★ Small quantity of petroleum ether,chloroform and methanolic extracts were dissolved in 5ml of chloroform separately, then these chloroform solution were subjected to Salkowski and Liebermann-Burchard test for detection of phytosterols.

Salkowski test

★ To 1ml of the above prepared chloroform solutions, few drops of concentrated sulphuric acid was added. Both the petroleum ether and methanolic extracts produced red colour in the lower layer. This showed the presence of phytosterols.

Liebermann-Burchard test

★ The chloroform solution was treated with few drops of concentrated sulphuric acid followed by 1ml of acetic anhydride solution. Green colour was produced in both petroleum and methanolic extracts, which showed the presence of phytosterols.

7. DETECTION OF SAPONINS

★ The extracts were diluted, with 20ml of distilled water and it was agitated in a graduated cylinder for 15minutes. A one centimetre layer of foam was produced in methanolic extract indicating the presence of saponins.

8. DETECTION OF TANNINS

Gelatin test

★ All the extracts were dissolved separately in minimum amount of water and filtered. To the filtrate, added 1ml of 1% solution of gelatin.

★ Petroleum ether and chloroform extracts gave white precipitate indicating the presence of tannins.

Ferric chloride test

★ The residues of all extracts were dissolved in water individually and to this a few drops of ferric chloride solution were added. Bluish black precipitate was produced, in chloroform and petroleum ether indicating the presence of tannins.

9. DETECTION OF PROTEIN AND AMINO ACIDS

★ Small quantities of allextracts were dissolved in few ml of water and they were subjected to millon's, Biuret and Ninhydrin tests.

Millon's test

★ The above prepared extracts were treated with millon's reagent and heated.
★ Red colour was produced with chloroform and methanolic extracts.

Biuret test

★ To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate were added.
★ Violet colour was produced it showed the presence of proteins and amino acid with methanolic and chloroform extracts.

Ninhydrin test

★ The above extracts were treated with Ninhydrin reagent.
★ Bluecolour was produced with chloroform and methanolic extracts.
★ The above three test indicated the presence of proteins and amino respectively.

10. DETECTION OF FLAVANOIDS

Shinoda's test

- ★ A small quantity of the extracts, were dissolved in alcohol and to this magnesium metal followed by concentrated hydrochloric acid was added in dropwise and heated.
- ★ A magenta colour was produced only in methanolic and chloroform extracts, indicating the presence of flavanoids.
- ★ Small quantity of the extracts were dissolved in chloroform, added small amount of ferric chloride and potassium ferricyanide.
- ★ A deep blue colour was produced in methanolic extract showed the presence of flavanoids.

11. DETECTION OF FLAVONES

- ★ With sodium, hydroxide solution, the methanolic extract gave yellow colour.
- ★ With concentrated sulphuric acid, methanolic extract gave orange colour.

Zinc, HCl Reduction test

- ★ To a small quantity of all three extracts, a pinch of zinc dust and few drops of concentrated hydrochloric acid was added. A Magenta colour was produced in methanolic extract.

12. Lead acetate solution test

- ★ To a small quantity of all extracts a few drops of 10% lead acetate solution was added.
- ★ Yellow precipitate was produced in methanolic extract, indicating the presence of flavones.

13. DETECTION ALKALOIIDS

- ★ A small quantity of the extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloidol reagents.

Mayer's test

★ All the extracts were mixed with Mayer's reagent (potassium mercuric iodide K_2Hgl_4) pale yellow precipitate was obtained with chloroform extract. Itsshowed the presence of alkaloids.

Dragondorff's test

★ All the extracts were mixed with Dragondorff's reagent (potassium bismuth iodide), orange red precipitate was obtained in chloroform extract, which indicate the presence of alkaloid.

Wagner's test

★ All extracts were mixed with Wagner's reagent (iodine in potassium iodide), reddish brown precipitate was obtained in chloroform extract, which showed the presence of alkaloids.

Hager's test

★ All the extracts mixed with hager's reagent (saturated aqueous solution of picric acid). Yellow crystalline precipitate was obtained in chloroformextract, which showed the presence of alkaloids.

★ From the above all test, it is confirmed that alkaloids are present in chloroform extract.

14. DETECTION OF COUMARINS

✦ A small quantity of all extracts were dissolved in alcohol and exposed to UV light. It was producedblue fluorescence.

- All the extracts were dissolved in alcohol and added with alcoholic ferric chloride, a bluish green colour was obtained in methanolic extract.

TABLE.NO.1

Data showing the preliminary phytochemical screening of the

pet.ether, chloroform,methanol extract of *Trichosanthes Cucumerina linn*

S.NO.	CONSTITUENTS	PET.ETHER EXTRACT	CHLOROFORM EXTRACT	METHANOL EXTRACT
1	CARBOHYDRATE	**-**	**+**	**+**
2	GLYCOSIDES	**+**	**+**	**+**
3	ALKALOIDS	**-**	**+**	**-**
4	FLAVANOIDS	**-**	**+**	**+**
5	FLAVONES	**-**	**-**	**+**
6	STEROIDS	**+**	**+**	**-**
7	PROTEINS& AMINO ACIDS	**-**	**+**	**+**
8	TANNIINS	**+**	**+**	**-**
9	SAPONINS	**-**	**+**	**+**
10	COUMARINS	**-**	**-**	**+**

+ indicates positive test results
- → indicates negative test results

These crude extracts were also investigated for the exhibition of some selective pharmacological activities.

ISOLATION, PURIFICATION AND IDENTIFICATION OF THE CONSTITUENTS

Based on the evidence of crude extract, 10 gms of Chlorofom extract was chromatographed over about 300 gms of silica gel column. The solvent used were petroleum ether, benzene, chloroform, ethyl acetate, methanol and their mixtures is in the order of increasing polarity.

The column was packed by using the suspension of silica gel in petroleum ether.

Each 100ml of the elutes were collected and concentrated. Each fraction was tested for the presence of various constituents and checked on TLC for number and type of constituents.

Details of Column Chromatography

Adsorbent	:	Silica Gel G (100 – 200 mesh)
Eluent	:	Petroleum ether –methanol – Chloroform (In Graduation)
Diameter of Column	:	3 cm
Length of Column packing	:	45 cm
Amount of chloroform extracts used	:	10 gms
Rate of elution	:	25 drops / min

Preparation of thin layer chromatography plate

About 30 gms of silica gel G was weighed, and it was shaken with 100 ml of distilled water to form a homogenous suspension. This suspension was poured into a TLC applicator, which was adjusted to 0.25 mm thickness.

The plates were kept in the hot air oven at 100°C for ½ hour to activate the silica Gel G. the plates were then stored in a dry atmosphere and used whenever required.

By using capillary tube, the fractions were spotted on TLC plates and the chromatogram was run in different solvent system. The compounds were developed related to their affinity towards different solvent system.

The different spot developed in each solvent system were identified in the iodine chamber and calculated the R_f Value.

$$R_F \text{ Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

TABLE.NO.2

DATA SHOWING THE COLOUMN CHROMATOGRAPHY OF CHLOROFORMIC EXTRACT OF *Trichosanthes cucumerina Linn*

Solvent	Ratio	Elute No.	Colour
Hexane	100 ml	1	Colourless
Hexane	100 ml	2	Colourless
Hexane	100 ml	3	Colourless
Hexane	100 ml	4	Colourless
Hexane	100 ml	5	Colourless
Hexane	100 ml	6	Colourless
Hexane : Benzene	90:10	7	Colourless
Hexane : Benzene	80:20	8	Colourless
Hexane : Benzene	70:30	9	Colourless
Hexane : Benzene	60:40	10	Yellow colour
Hexane : Benzene	50:50	11	Yellow colour
Benzene	50ml	12	LightYellow colour
Benzene	50ml	13	LightYellow colour
Benzene	50ml	14	LightYellow colour
Benzene : Chloroform	90:10	15	LightYellow colour
Benzene : Chloroform	80:20	16	Yellow colour
Benzene : Chloroform	70:30	17	Yellow colour
Benzene : Chloroform	60:40	18	Yellow colour
Benzene : Chloroform	50:50	19	Yellow colour
Chloroform	100ml	20	Yellow colour
Chloroform	100ml	21	Yellow colour
Chloroform:EtoAc	90:10	15	LightYellow colour
Chloroform:EtoAc	80:20	16	Yellow colour
Chloroform:EtoAc	70:30	17	Yellow colour
Chloroform:EtoAc	60:40	18	Yellow colour
Chloroform:EtoAc	50:50	19	Yellow colour
Ethyl acetate[150ml]	50ml	27	Dark green colour
	50ml	28	Dark green colour
	50ml	29	Dark green colour
EtoAc: Methanol	90:10	30	Blackish green colour
EtoAc: Methanol	80:20	31	Blackish green colour
EtoAc: Methanol	70:30	32	Blackish green colour
EtoAc: Methanol	60:40	33	Blackish green colour
EtoAc: Methanol	50:50	34	Blackish green colour

TABLE.NO.3

DATA SHOWING COLOUMN CHROMOTOGRAPHY ANALYSIS

FROM CHLOROFORM EXTRACT

FRACTION NO	SOLVENT(TLC)	RATIO	Rf VALUE	CRYSTALLISATION SOLVENT	COLOUR	COMPOUND NAME
29,30,31,32	EtoAc :CH_3OH	9:1	0.6932	Absolute alcohol	Green	TCA
25,26,27,28	Hex : EtoAc	6:4	0.4210	Absolute alcohol	Greenish violet	TCB
22,23,24	Hex : EtoAc	9.5:0.5	0.6930	Chloroform	Light brown	TCC
20,21	$CHCl_3$:EtoAc	8:2	0.4528	Chloroform	Yellowish brown	TCD
16,17,18,19	$CHCl_3$:EtoAc	8:2	0.5579	Chloroform	Yellowish green	TCE
10,11,12	Pet.ether:Benzene	5:5	0.6829	Hexane	Yellow	TCF

IDENTIFICATION OF ISOLATED COMPOUNDS

I.COMPOUND TCA

The compound was isolated from the dried aerial part of ***TRICHOSANTHES CUCUMERINA***.*Linn.*

1. Physical Examination:

Colour	:	Green
State	:	Semi solid
Yield obtained	:	100mg
Solubility	:	Absolute alcohol, Chloroform
Melting point	:	180ºC - 220ºC

2. T.L.C system

Adsorbent	:	Silica gel
Solvent system	:	EtoAc : Methanol (9:1)
Identification	:	UV Lamp
Absorbance	:	270nm
Rf value	:	0.6932

3.CHEMICAL TEST:

❖ **Detection of *Steroidal glycosides***

***Phytosterols*.**

Small quantity of compound TCA was dissolved in 5ml of chloroform separately. Then this solution was subjected to Salkowski and Liebermann – Burchard test for the detection of phytosterols.

a) Salkowski test:

To 1 ml of the above prepared chloroform solutions, few drops concentrated sulphuric acid was added. Itproduced red colour in the lower layer showed the presence of phytosterols.

b) Liebermann – Burchard test:

The above chloroform solution was treated with the few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. Green colour was produced indicating the presence of phytosterols.

Detection of *Glycosides*

a) Legal's test:

The compound TCA 1 ml of pyridine and few drops of sodium nitro prusside solution were added, and then it was made alkaline with sodium hydroxide.

Pinkto yellow colour was obtained, it showed the presence of glycosides.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND TCA

TABLE NO: 4

IR SPECTRUM DATA

S.NO	FREQUENCY CM^{-1}	GROUPS ASSIGNED
1	3853.65	Aromatic C-H Stretching
2	3430.64	May be due to O-H Stretching
3	3014.58	May be due to O-H Stretching
4	2925.11	May be dueto C-H Stretching
5	2859.19	May be dueto C-H Stretching
6	1713.49	May be due to C=O Stretching
7	1443.95	May be due to Sp3 C-H Bending
8	1367.06	May be due to Sp3 C-H Bending
9	1220.36	May be due to C-O Stretching
10	1091.72	May be due to C-N Vibration
11	767.13	May be due to C-H Bending(opposite)
12	671.58	May be due to N-H Bending(opposite)

5) ^{1}H NMR spectra of compounds:

^{1}H NMR was taken using $CDCl_3$ in 300mHZ. TMS as standard as shown in the table and the spectrum was attached.

TABLE: 5

^{1}H NMR SPECTRUM DATA

SNO	SIGNAL◊VALUES (PPM)	GROUPS ASSIGNED
1	0.781-0.993	May be due to CH3 proton
2	1.179	May be due to CH3 proton
3	1.470-1.602	May be due to CH proton attached to alkyl group
4	1.525	May be due to CH proton attached to alkyl group
5	1.895-1.980	May be due to alylic proton (C=C)
6	2.222-2.266	May be due to CH2 proton adjacent to C=O
7	2.713-2.748	May be due to CH2 proton adjacent to C=O
8	3.179-3.797	May be due to OH (Ester proton)
9	3.826-3.993	May be due to Ester proton
10	4.O29-4.252	May be due to acyclic non conjucated bond
11	5.042-5.462	May be due to CH2 proton attached to ethylinic bond
12	7.202	May be due to aromatic nature

II.COMPOUND TCB

The compound was isolated from the dried aerial parts of ***TRICHOSANTHES CUCUMERINA.****Linn.*

1. Physical Examination:

Colour	:	Greenish violet
State	:	Semi solid
Yield obtained	:	120mg
Solubility	:	Absolute alcohol
Melting point	:	190ºC - 230ºC

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Hexane : EtoAc (6:4)
Identification	:	UV Lamp
Absorbance	:	260nm
Rf value	:	0.4210

3.CHEMICAL TEST:

Detection of *Phytosterols*.

Small quantity of the compound TCB was dissolved in 5ml of chloroform separately. Then this solution was subjected to Salkowski and Liebermann – Burchard test for the detection of phytosterols.

c. Salkowski test:

To 1 ml of the above prepared chloroform solution, few drops concentrated sulphuric acid was added. Itsproduced red colour in the lower layer. It showed the presence of phytosterols.

d) Liebermann – Burchard test:

The above chloroform solution was treated with the few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. Green colour was produced in indicating the presence of phytosterols.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND TCB

TABLE NO:6

IR SPECTRUM DATA

S.NO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3946.26	Aromatic C-H Stretching
2	3423.92	May be due to O-H Stretching
3	2926.16	May be due to O-H Stretching

4	2865.02	May be due to C-H Stretching
5	2668.58	May be due to C-H Stretching
6	1710.17	May be due to C=O Stretching
7	1453.71	May be due to Sp3 C-H Bending
8	1378.44	May be due to Sp3 C-H Bending
9	1219.76	May be due to C-O Stretching
10	764.84	May be due to C-H Bending(Opposite)
11	666.78	May be due to N-H Bending(Opposite)

5) ^{1}H NMR spectra of compounds:

^{1}H NMR was taken using $CDCl_3$ 300mHZ. TMS as standard as shown in the table and the spectrum was attached.

^{1}H NMR SPECTRUM DATA

TABLE NO:4

SNO	SIGNAL(σ)VALUES PPM	GROUPS ASSIGNED
1	0.411-0.916	May be due to CH3 proton
2	1.500-1.599	May be due to C-H proton attached to alkyl group(C=C)
3	1.725-1.802	May be due to C-H proton attached to alkyl group(C=C)
4	1.933-2.018	May be due to C-H proton attached to alkyl group(C=C)
5	2.228-2.277	May be due to CH2 proton adjacent to C=O
6	2.704-2.741	May be due to CH2 proton adjacent to C=O
7	3.001	May be due to CH2 proton adjacent to C=O
8	3.269-3.300	May be due to CH2 proton attached to O-R
9	3.419-3.620	May be due to CH2 proton attached to O-R
10	3.743-3.854	May be due to OH
11	4.062-4.139	May be due to acyclic non conjucated bond
12	4.346-4.465	May be due to Nitro group
13	5.004-5.128	May be due to CH2 proton attached to

		ethylinic bond
14	5.210-5.321	May be due to CH2 proton attached to ethylinic bond
15	6.014-6.194	Aromatic nature
16	7.165	Aromatic nature
17	7.745-7.856	Aromatic nature
18	8.380-8.537	Aromatic nature

^{13}CNMR SPECTRUM DATA

COMPOUND TCB

TABLE NO : 7

S.NO	**VALUES(σ)**
1	12.08
2	12.98
3	14.29
4	19.37
5	20.57
6	22.63-23.09
7	24.42-25.63
8	27.91
9	29.10-30.33
10	31.94
11	32.62
12	33.98
13	36.64
14	37.39
15	39.36
16	39.80
17	127.12
18	127.78
19	128.296
20	129.747-130.259
21	131.96

III.COMPOUND TCC

The compound was isolated from the dried aerial parts of ***TRICHOSANTHES CUCUMERINA***.*Linn.*

1. Physical Examination:

Colour	:	Yellowish brown
State	:	Semi solid
Yield obtained	:	50mg
Solubility	:	Chloroform
Melting point	:	140ºC - 160ºC

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Hexane : EtoAc (9.5 : 0.5)
Identification	:	UV Lamp
Absorbance	:	280nm
Rf value	:	0.6931

3.CHEMICAL TEST:

❖ **Detection of *Flvanoids***

a) SHINODA'S TEST:

A small quantity of the compound TCC was dissolved in alcohol and to this magnesium metal followed by concentrated HCL was added in dropwise and heated.Mejenta colour was produced indicating the presence of Flavanoids

b)The sample mixed with chloroform and exposed to UV light. It produced pink colour.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND TCC

TABLE NO: 8

IR SPECTRUM DATA

S.NO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3931.02	Aromatic C-H Stretching
2	3414.84	May be due to O-H Stretching
3	2923.47	May be due to C-H Stretching
4	2438.41	May be due to C-H Bending
5	1715.67	May be due to C=O Stretching
6	1445.25	May be due to Sp3 C-H Bending
7	1367.83	May be due to Sp3 C-H Bending
8	1222.60	May be due to C-O Stretching
9	1094.84	May be due to C-N Bending(opposite)
10	764.60	May be due to C-H Bending(opposite)

IV.COMPOUND TCD

The compound was isolated from the dried aerial part of ***TRICHOSANTHES CUCUMERINA****.Linn.*

1. Physical Examination:

Colour : Yellowish green

State : Semi solid

Yield obtained	:	60mg
Solubility	:	Chloroform
Melting point	:	120ºC - 140ºC

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Chloroform : EtoAc (8:2)
Identification	:	UV Lamp
Absorbance	:	265nm
Rf value	:	0.4528

3.CHEMICAL TEST

❖ **Detection of *Flavones:***

a) With sodium hydroxide solution the compound TCD produced yellow colour.
b) Compound TCD were produced orange colour with concentrated sulphuric acid.

c) Zinc, HCl reduction test

To a small quantity of the compound a pinch of zinc dust was added. Then added a few drops of concentrated hydrochloric acid. Magenta colour was produced in after a few minutes.

d) Lead acetate solution test:

To a small quantity of the compounds a few drops of 10% lead acetate solution was added. Yellow precipitate was produced in indicating the presence of flavones.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND TCD

TABLE NO: 9

IR SPECTRUM DATA

SNO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3958.34	Aromatic C-H Stretching
2	3848.20	Aromatic C-H Stretching
3	3744.10	Aromatic C-H Stretching
4	3420.80	May be due to O-H Stretching
5	3094.61	May be due to Sp3 C-H Stretching
6	2920.30	May be due to C-H Stretching
7	2467.33	May be due to C-H Bending
8	1643.58	May be due to C=C Stretching
9	1453.09	May be due to Sp3 C-H Bending
10	1381.57	May be due to Sp3 C-H Bending
11	1219.14	May be due to C-O Stretching(aryl,alkyl,ether)
12	1092.24	May be due to C-N Bending(opposite)
13	770.11	May be due to C-H Bending(opposite)

V.COMPOUND TCE

The compound was isolated from the dried aerial part of ***TRICHOSANTHES CUCUMERINA***.*Linn.*

1. Physical Examination:

Colour	:	Yellowish brown
State	:	Semi solid
Yield obtained	:	100mg
Solubility	:	Chloroform
Melting point	:	145ºC - 155ºC

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Chloroform : EtoAc (8:2)
Identification	:	UV Lamp
Absorbance	:	280nm
Rf value	:	0.5579

3.CHEMICAL TEST

Detection of *Flavanoids*

a) SHINODA'S TEST:

A small quantity of the compound TCE were dissolved in alcohol and to this magnesium metal followed by concentrated HCL was added dropwise and heated. Megenta colour was produced indicating the presence of Flavanoids

b) The sample mixed with chloroform and exposed to UV light. It produced pink colour.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND TCE

TABLE NO: 10

IR SPECTRUM DATA

S.NO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3369.60	May be due to O-H Stretching
2	2918.08	May be due to Sp3 C-H Stretching
3	2849.88	May be due to Sp3 C-H Stretching

4	1734.98	May be due to C=O Stretching
5	1463.20	May be due to Sp3 C-H Bending
6	1260.21	May be due to C-O Stretching(aryl,alkyl,ether)
7	1092.77	May be due to C-N Bending(opposite)
8	1022.82	May be due to C-N Bending(opposite)
9	801.56	May be due to C-H Bending(opposite)
10	721.90	Aromatic

5) ^{1}H NMR spectra of compounds:

^{1}HNMR was taken ($CDCl_3$) 300mHZ. TMS as standard as shown in the table and the spectrum was attached.

COMPOUND TCE

TABLE : 11

^{1}H NMR SPECTRUM DATA

SNO	SIGNAL(σ)VALUES PPM	GROUPS ASSIGNED
1	0.763-0.915	May be due to CH3 proton
2	1.184-1.229	May be due to CH2 proton attached to alkyl group(C=C)
3	1.529-1.677	May be due to C-H proton attached to alkyl group(C=C)
4	1.931-1.970	May be due to C-H proton attached to alkyl group(C=C)
5	2.220-2.307	May be due to CH2 proton adjacent to C=O
6	2.684-2.758	May be due to CH2 proton adjacent to C=O

7	3.184.3.212	May be due to CH2 proton attached to O-R
8	3.493-3.669	May be due to CH2 proton attached to O-R
9	3.981-4.527	May be due to aromatic CH2OH proton(Ester)
10	5.046-5.294	May be due to CH2 proton attached to ethylinic bond
11	7.196	Aromatic nature

V.COMPOUND TCF

The compound was isolated from the dried aerial part of ***TRICHOSANTHES CUCUMERINA***.*Linn.*

1. Physical Examination:

Colour	:	Yellow
State	:	Semi solid
Yield obtained	:	100mg
Solubility	:	Chloroform
Melting point	:	150ºC - 165ºC

2. T.L.C SYSTEM

Adsorbent	:	Silica gel
Solvent system	:	Chloroform : EtoAc (8:2)
Identification	:	UV Lamp
Absorbance	:	290nm
Rf value	:	0.5579

3.CHEMICAL TEST

Detection of *Flavanoidolglycosides:*

Test for flavanoid

a) SHINODA'S TEST:

A small quantity of the compound TCF were dissolved in alcohol and to this magnesium metal followed by concentrated HCL was added dropwise and heated. Megenta colour was produced indicating the presence of Flavanoids.

b)The sample mixed with chloroform and exposed to UV light. It produced pink colour.

Detection of *Glycosides*

e) Legal's test:

To the compound added 1 ml of pyridine and few drops of sodium nitro prusside solution were added, and then it was made alkaline with sodium hydroxide.Pink to yellow colour was obtained, it showed the presence of glycosides.

4) IR ANALYSIS

Media: KBr

The spectrum attached. IR peaks and the groups assigned are shown in the table.

COMPOUND TCF

TABLE NO: 12

IR SPECTRUM DATA

S.NO	FREQUENCY(CM^{-1})	GROUPS ASSIGNED
1	3753.67	May be due to Aromatic C-H Stretching
2	3729.14	May be due to Aromatic C-H Stretching
3	2923.12	May be due to Sp^3 C-H Stretching
4	2853.83	May be due to Sp^3 C-H Stretching
5	2371.04	Extended resonance
6	2340.97	Extended resonance
7	1655.05	May be due to C=O Stretching
8	1459.83	May be due to Sp^3 C-H Bending
9	1020.83	May be due to C-N Stretching (opposite)

5) ^{1}H NMR spectra of compounds:

^{1}HNMR was taken using $CDCl_3$300mHZ. TMS as standard as shown in the table and the spectrum was attached.

COMPOUND TCF

TABLE :13

H NMR SPECTRUM DATA

S.NO	SIGNAL(σ)VALUES PPM	GROUPS ASSIGNED
1	0.811-0.915	May be due to CH_3 proton
2	1.183-1.204	May be due to CH_2 proton attached to alkyl group(C=C)
3	1.436	May be due to CH_2 proton attached to alkyl group(C=C)
4	1.543	May be due to C-H proton attached to alkyl group(C=C)
5	1.926-2.017	May be due to C-H proton attached to alkyl group(C=C)
6	2.148-2.307	May be due to CH_2 proton adjacent to C=O
7	2.833-3.081	May be due to CH_2 proton adjacent to C=O
8	3.184-3.212	May be due to CH_2 proton attached to O-R
9	3.477-3.690	May be due to CH_2 proton attached to O-R
11	3.981-3.996	May be due to Ester proton
12	4.044-4.519	Aromatic alchoholic or phenolic proton
13	5.180-5.293	May be due to CH2 proton attached to ethylinic bond
14	7.196	Aromatic nature

DIURETIC ACTIVITY[65-75]

Introduction

Diuretics increase urine flow by action on the kidney. Most agents affect water balance indirectly by altering electrolyte reabsorption or secretion.

Diuretics are drugs that inhibit sodium transport which results in a concomitant loss of water and reduction of extracellular volume. The terms saluretic or natriuretic appropriate for this while the old name diuretic should mean a drug that increases urinary volume.

Substances increasing urine flow are classified into two groups.

1. Agents which increase renal plasma flow and glomerular filtration rate.
2. Agents which increase solute excretion of the glomerular filtrate and tubular fluid.

Historically, the classification of diuretics was based on a mosaic of ideas such as site of action(loop – diuretics), efficacy (high ceiling diuretics), chemical structure (thiazide – like diuretics), effects on potassium excretion (potassium – sparing diuretics) etc.

Factors governing diuretics

1. Glomerular filtration

   This is mainly determined by

   a) The rate of flow through the kidney tissue.
   b) Number of functioning renal units at a time.
   c) Osmotic pressure of plasma proteins
   d) Hydrostatic pressure in the glomeruli
   e) Permeability of the filtering membrane
2. Tubular reabsorbtion
3. Tubular secretion

Properties of an ideal diuretic

A good diuretic drug should have following properties:

a) Should be safe.
b) Should promote excretion of Na and Cl in equal amounts.
c) Should have rapid onset of action.
d) Should have long duration action.
e) Should be orally administered.
f) Should have minimum side effects like acidosis, alkalosis or excretion of HCO_3^-

EXPERIMENTAL

Purpose and Rationale

A method for testing diuretic activity in rats has been described by Lipschitz et al (1943). The test was based on water and sodium excretion in test animals and were compared to rats treated with a high dose of urea. The "Lipschitz – value" is the quotient between excretion by test animals ad excretion by the urea control.

Requirements

Animal	-	Male Albino wistar rats
Drugs and Chemicals	-	Normal saline, Furosemide (std), Pet ether, chloroform & Methanolic extract of Trichosanthes cucumerina Linn.
Instrument used	-	Metabolic cage

Procedure

Normally healthy male albino wistar rats, weighing between 180 – 200gms were used for this study. The animals were divided into 5 groups

consisting of six animals in each group. These animals were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the 24 hrs urine sample. Stainless-steel sieves are placed in the funnel to retain faeces and to allow the urine to pass. The rats were placed in the standard diet and water fifteen hours prior to the experiment food and water were withdrawn. The dosage of the drug administered to different groups as follows.

Treatment protocol

Group I

A control group received orally 10ml/kg body weight of normal saline.

Group II

The standard group received orally 25mg/kg body weight of furosemide (Loop diuretics).

Group III

The treatment group received orally 100mg/kg body weight of petroleum ether extract of Trichosanthes cucumerina Linn.

Group IV

The treatment group received orally 100mg/kg of body weight of chloroform extract of Trichosanthes cucumerina Linn.

Group V

The treatment group received orally 100mg/kg of body weight of methanolic extract of Trichosanthes cucumerina Linn.

The above extracts were dissolved in 2ml of sterile water and administered through orally. The urine sample were measured at 24 hrs. After collection of urine, the urine was analysed by flame photometer for evaluation of various electrolyte like Na^+, 2K, and Cl concentration was evaluated by titration

with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate as an indicator. The role of electrolytes as tabulated.

Electrolyte excretion and Diuretic activity of various extracts of

Trichosanthes cucumerina Linn.

Groups	Treatment	Dose	Urine	Electrolyte	Excretio	Meq/lit	$Na^{+/}$

			volume	Na^+	n K^+		K^+
Group I	Normal Control	10ml/kg	8.8 ± 2.30	61.93 ± 3.14	49.50 ± 2.30	23.70 ± 2.31	0.012
Group II	Standard Control	25mg/kg	17.4 ± 3.40	121.80 ± 5.60	22.86 ± 1.40	78.26 ± 3.96	5.32
Group III	Treatment Control	100mg/kg Pet.ether	9.3 ± 2.16	80.40 ± 3.90	36.30 ± 1.95	59.21 ± 2.60	2.21
Group IV	Treatment Control	100mg/kg Chloroform extract	10.4 ± 2.40	99.26 ± 4.10	39.10 ± 2.05	49.05 ± 1.95	2.53
Group V	Treatment Control	100mg/kg Methanol extract	12.3 ± 2.90	108.80 ± 4.46	42.66 ± 2.15	42.11 ± 1.30	2.55

Values are expressed as Mean ± SEM

Values are find out by using one way ANOVA followed by Neuman Keul's multiple range test.

Values were significantly different from normal control at $P < 0.01$

Results

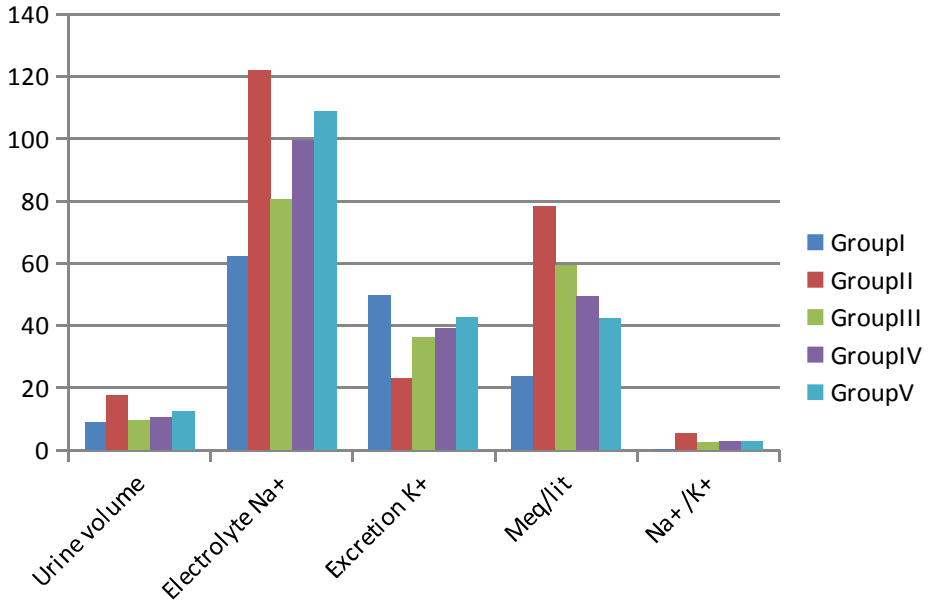
Diuretic Activity

Increase in urine output a sufficient index for assessing the diuretic effect through estimating the urinary concentration of Ion like Na+, K+, Cl- etc., may reveal in specific the Ion responsible for the diuretic activity.

The results reveal that electrolyte excretions & diuretic activity of various extract of Trichosanthes cucumerina treatment posseses. Significantly diuretic activity at $P < 0.01$. But methanolic extract produced more ssignificant fall in potassium excretion compared to control.

Electrolyte excretion and Diuretic activity of various extracts of

Trichosanthes cucumerina Linn



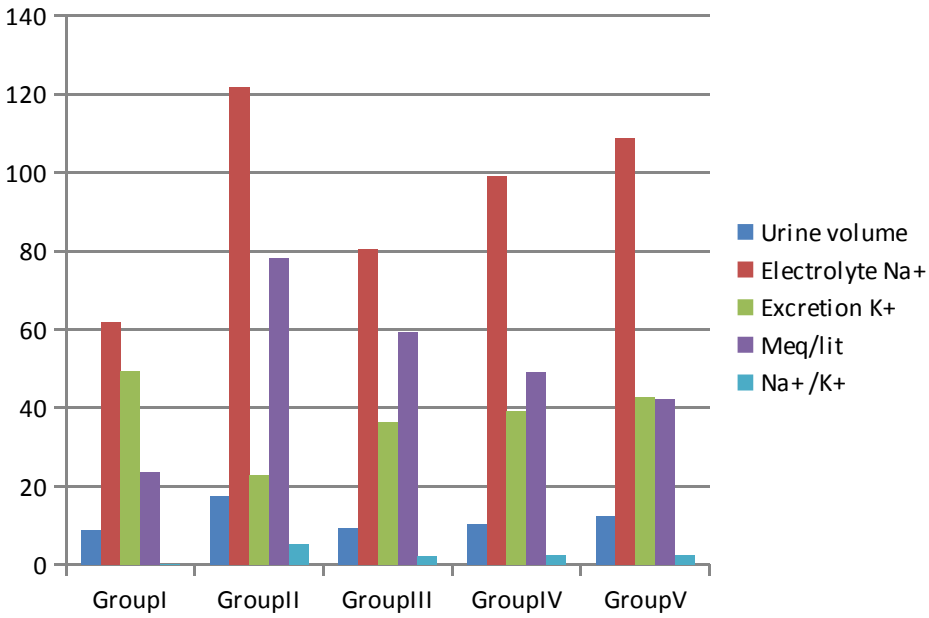


TABLE VALUES SHOWS THAT METHANOLIC EXTRACT OF TRICHOSANTHES CUCUMERINA LINN EXHIBITED SIGNIFICANT DIURETIC ACTIVITY THAN OTHER TWO EXTRACTS.

ANTHELMINTIC ACTIVITY[75-84]

INTRODUCTION

Anthelmintics drugs are that which expel parasitic worms from the body, by either stunning or killing them. Helminthes infections are now being recognized as cause of many acute as well as chronic health's among the various human beings as well as cattle. More than half of the population of the world suffers from infection of one or the other and majority of cattle's suffers from worm infections. Treatment with an anthelmintic drug kills worms whose genotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. Intestinal worm infections in general are more easily treated than those in other locations in the body. Because the worms need not be killed by the drug and the drug need not be absorbed when given by mouth, there is usually a wider margin of safety than with drugs for worm infections in other sites. Traditional system of medicine reports the efficacy of several natural plants in eliminating worms the present work was conceived by us to evaluate the anthelmintic activity of polyherbal preparation containing three herbs viz., *Erythrina indica* bark, *Fumaria officinalis* stem and *Cleome viscosa* leaves.

MATERIALS AND METHODS

Animal	-	Pheretima posthuma worms (earth worms)
Drugs and Chemicals	-	Normal saline, Albendazole (std), Pet ether, chloroform & Methanolic extract of Trichosanthes cucumerina Linn.

Standard Drug

Albendazole is taken as standard drug and the concentration of the standard drug was prepared in 1% gum acacia in normal saline to give 0.5, 0.75 and 1% concentration.

Experimental Model

The anthelmintic activity was evaluated on earth worms by the method of Mathew et.al and Dash et.al was followed. The assay was performed on adult Indian earth worm due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Equal sized (8 ±1cm) worms were selected for the study. The worms were washed with normal saline to remove all the extrageneous matter. Eight groups of approximately equal size Indian earth worms consisting of six earth worms in each group were released into 50ml of desired formulation. Each group was treated with one of the following. The first group served as control (received 1% gum acacia in normal saline), second group served as standard (received Albendazole 10mg/ml), third, fourth and fifth groups were (received pet ether, chloroform and methanolic extracts of 10mg, 25mg, in 1% gum acacia in normal saline respectively). The above prescribed dose were prepared and poured into respective labeled Petri plates and the volume was made up to 50ml with normal saline. The standard Albendazole (10mg/ml) and the test pet ether, chloroform and methanolic extracts (10mg, 25mg /ml) were evaluated for anthelmintic activity.

OBSERVATIONS STUDY

Observations were made for the time taken for paralysis and death of individual worms. The mean paralysis time and mean lethal time of standard of each extract was recorded. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when worms lost their motility followed with fading away of their body colour. Death was also confirmed by dipping the worm in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased. The results were shown in Table.

Treatment protocol

Group I

A control group received orally 10ml/kg body weight of normal saline.

Group II

The standard group received orally 10mg/ml of Albendazole

Group III

The treatment group received orally 10mg/ml of petroleum ether extract of Trichosanthes cucumerina Linn.

Group IV

The treatment group received orally 25mg/ml of petroleum ether extract of Trichosanthes cucumerina Linn.

Group V

The treatment group received orally 10mg/ml of chloroform extract of Trichosanthes cucumerina Linn.

Group VI

The treatment group received orally 25mg/ml of chloroform extract of Trichosanthes cucumerina Linn.

Group VII

The treatment group received orally 10mg/ml of methanolic extract of Trichosanthes cucumerina Linn.

Group VIII

The treatment group received orally 25mg/ml of methanolic extract of Trichosanthes cucumerina Linn**.**

Anthelmintic activity of various extracts

Trichosanthes cucumerina Linn

Groups	Treatment	Conc. used (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
I	Control	1% Acacia in Normal Saline	-	-
II	Albendazole	10	36.60±0.64	61.92±1.66
III	Pet.Ether	10	48.09±0.45	75.46±1.13
IV	Pet.Ether	25	34.75±0.41	67.07±2.05
VI	Chloroform extract	10	46.16±0.46	70.73±2.08
VII	Chloroform extract	25	30.04±0.47	50.11±1.40
VII	Methanol extract	10	40.01±0.51	63.06±1.19
VIII	Methanol extract	25	23.75±0.77	38.44±1.48

Values are expressed as mean ± S.E.M (n=6) Control worms were alive up to 24 hours of the experiment.

The result obtained was compared with the standard drug Albendazole. Pet ether, chloroform and methanol extracts of *Trichosanthes cucumerina Linn* aerial parts exhibited dose dependent and significant anthelmintic activity compared

with standard drug Albendazole on Indian earth worms. Among the extracts methanolic extract required least time to causes paralysis and death of the earth worm followed by chloroform and Pet ether extracts.

Statistical Analysis

Results are expressed as mean ± S.E.M were evaluated by one way ANOVA followed by Newman Kew's multiple range tests. Values of $P < 0.001$ were considered statistically significant.

Results and Discussion

Trichosanthes cucumerina Linn produced a potent anthelmintic activity against the pheretima posthuma when compared with reference standard Albendazole ($P < 0.001$). The methanolic extract showed significant activity than pet ether and chloroform extracts. This activity was Concertration dependent. The Potency was found to be inversely proportional to the time taken for paralysis and death of the worms.

Anthelmintic activity in Methanolic extract

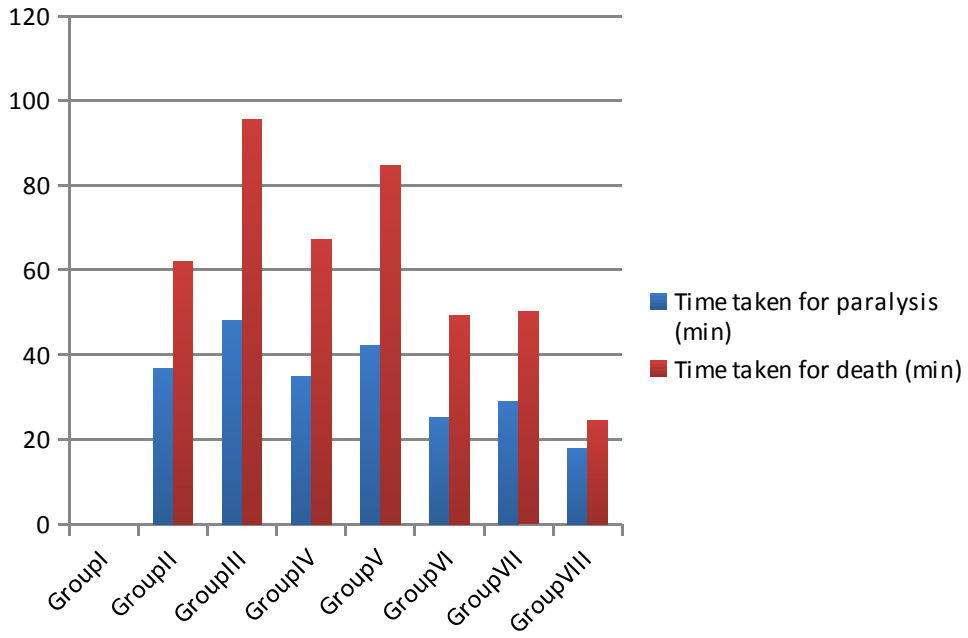
Stage 1 : Normal

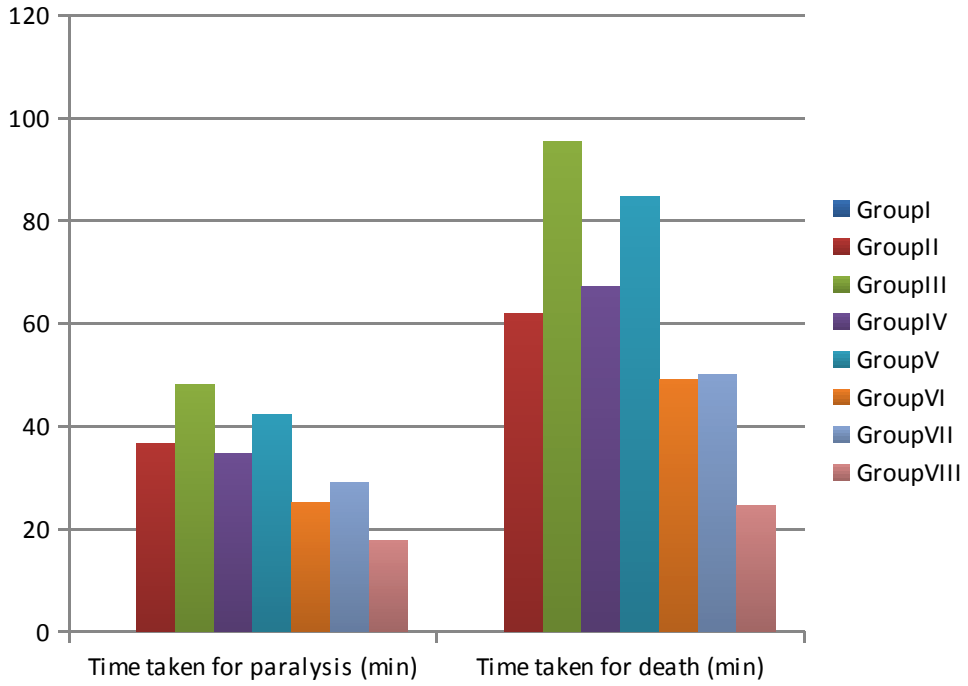
Stage 2 : Paralysis

Stage 3 : Death

Anthelmintic activity of various extracts

Trichosanthes cucumerina Linn





RESULTS AND DISCUSSIONS

Several chemical constituents and physiochemical properties of Trichosanthes cucumerina Linn , I have decided to work on the whole plant , since its widely used in the indigenous medicine. The dried whole plant were made in to a coarse powder and extracted with **Pet.ether AR, chloroform AR, and methanol AR**. Solvents in the order of increasing polarity.

The physiochemical screening of Trichosanthes cucumerina linn was to study, isolate and characterization of chemical constituents and pharmacological activity. During my investigation the above mentioned solvents, extracts showed the presence of **carbohydrate, glycoside, saponins, tannins, flavanoids, phytosterol, flavones coumarins and alkaloid.**

Regarding the isolation procedure is concerned the chloroform extract was subjected to column chromatography using the silica gel 100-200 mesh. During my investigation six compounds were isolated namely **TCA, TCB, TCC, TCD, TCE, TCF**. These compounds were eluted with the solvents in the increasing order of polarity like Pet ether, benzene, chloroform, ethyl acetate and methanol.

The compound **TCA** showed green colour which is semisolid in state. The melting point was 180-220ºc which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using ethyl acetate : methanol (9:1) having the UV absorbance of 270 to nm. The Rf value was found to be 0.6932. The IR data showed the frequency at 3853,3430,2925,1713,1443,1220,1091,767 cm^{-1} and ^{1}HNMR showed the signals at 0.993,1.470,1,525,1.895,2.266,3.179,5.462,7.202 ppm showed that this compound may be **steroidol glycoside** type, which was conformed by chemical test.

The compound **TCB** showed greenish violet colour which is semisolid in state. The melting point was 190-230ºc which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using ethyl actate : hexane (4:6) having the UV absorbance of 260 nm. The Rf value was found to be 0.4210. The

IR data showed the frequency at 3946, 3423, 2865, 1710, 1453, 1219, 666 cm^{-1} and ^{1}HNMR showed the signals at 0.916, 1.599, 2.018, 2.277, 3.269, 3.743 δppm and ^{13}C NMR showed the value at 12.08, 12.98, 14.29, 19.37, 22.63, 25.62, 29.10, 32.62, 36.64, 39.36, 127.78, 130.25. This Suggested that this compound may be **phytosterols** type, which was conformed by chemical test.

The compound **TCC** showed yellowish brown in colour which is semisolid in state. The melting point was 140-160ºc which is soluble in absolute alcohol and double chloroform. The TLC showed the single spot using hexane : ethanol (9.5:0.5) , Rf value was found to be 0.6931 having the UV absorbance of 280nm.The IR data showed the frequency at 3931,3414,2923,1715,1445,1222,764 cm^{-1} suggested that these compound may be a **flavanoid** type, which was conformed by chemical test.

The compound **TCD** showed yellowish brown colour which is semisolid in state. The melting point was 120-140ºc which is soluble in chloroform. The TLC showed a single spot using chloroform : ethanol (8:2), Rf value was 0.4528 having the UV absorbance of 265 nm. The IR data showed the frequency at 3958, 3420, 3094, 2920, 2467, 1643, 1453, 1219, 1092 and 770 cm^{-1}. This suggested that this compound may be a **flavone** type , which was conformed by chemical test.

The compound **TCE** showed yellowish brown in colour which is semisolid in state. The melting point was 145-155ºc which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using chloroform : ethanol (9:1) having the UV absorbance of 280 nm. The Rf value was found to be 0.5579. The IR data showed the frequency at 3369, 2918, 1734, 1463, 1260, 1022, 801 cm^{-1} and ^{1}HNMR showed the signals at 0.763, 1.229, 1.529, 3.212, 3.981, 5.294, 7.196 δppm. This Suggested that this compound may be a **phytostero**l type , which was conformed by chemical test.

The compound **TCF** showed yellow in colour which is semisolid in state. The melting point was 150-165ºc which is soluble in chloroform. The TLC showed a single spot using chloroform : ethanol (8:2) having the UV absorbance

of 290 nm. The Rf value was found to be 0.4230. The IR data showed the frequency at 3753, 2923, 1655, 1459, 1020 cm^{-1} and ^{1}HNMR showed certain signals at 0.811, 1.183, 1.204, 1.543, 2.148, 3.081, 3.212, 4.519, 5.298, 7.196 δppm. This Suggested that this compound may be **flavanoid glycosides** type, which was conformed by chemical test.

Regarding pharmacological activity the whole plant extract revealed the **Diuretic activity** and **Anthelmintic activity** on Pet ether, chloroform and methanolic extracts of Trichosanthes cucumerina Linn. The methanolic extract of Trichosanthes cucumerina linn exhibited significant Diuretic activity than the other two extracts.

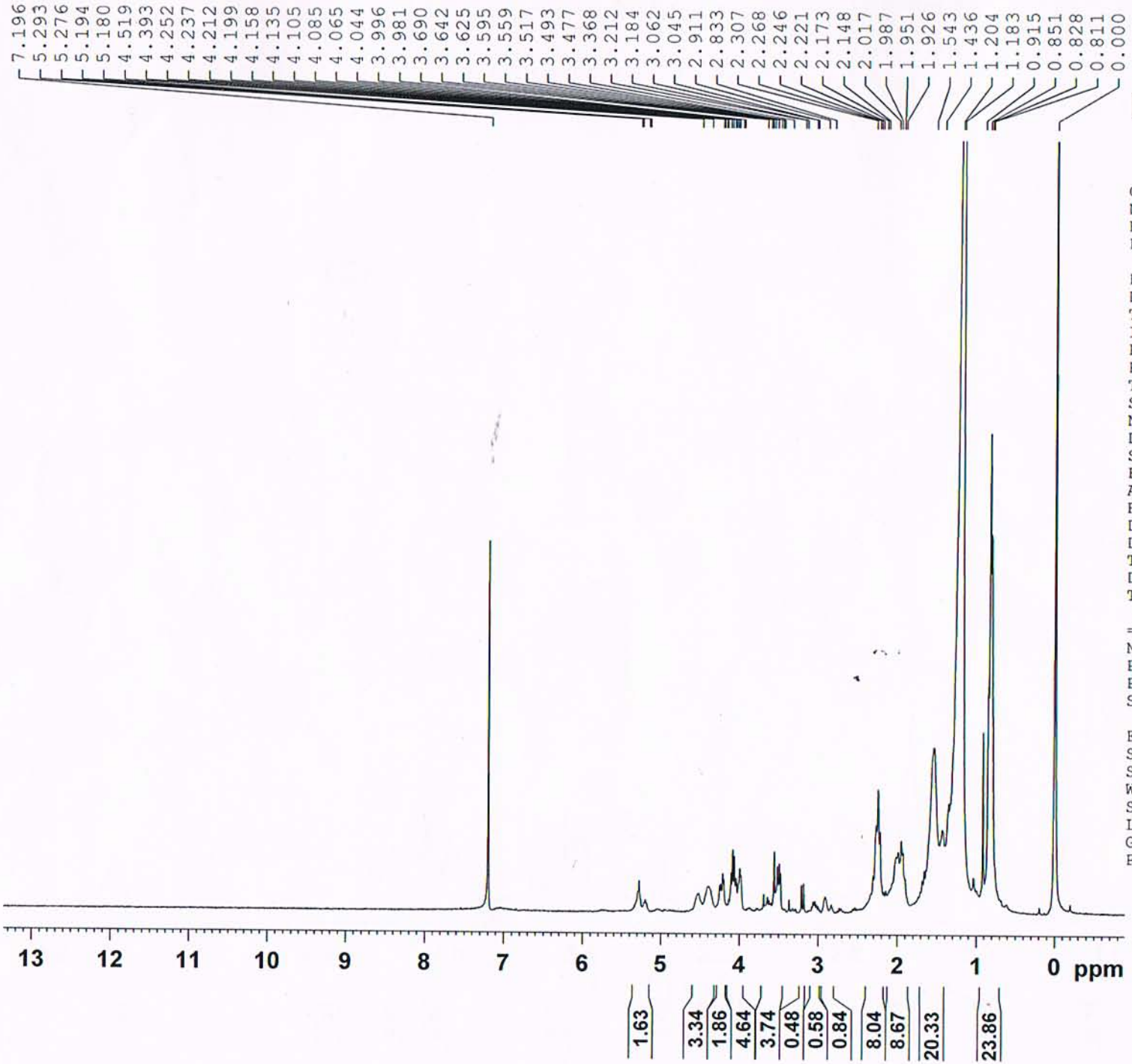
As for as the anthelmintic activity is concerned, the extracts of Trichosanthes cucumerina Linn, the methanolic extract exhibited significant **Anthelmintic activity** than other two extracts.

CONCLUSION

Since a number of phyto constituents are present in these extracts, further studies were planned to isolate and characterize the compound respectively in the pharmacological activities the protocol studies of petroleum ether, Chloroform and Methanolic extracts showed almost equipotent significance, when compared to standard.

The natural and synthetic phyto constituents play a vital and a major role in pharmacological, clinical studies, which showed an important impact in preparing natural product based libraries for combinational chemistry.

Henceforth, at docimasy, these therefore provide a basis for detailed investigation of various parameters on therapeutic efficacy, which undergo to obtain excellent use in indigenous medicine.



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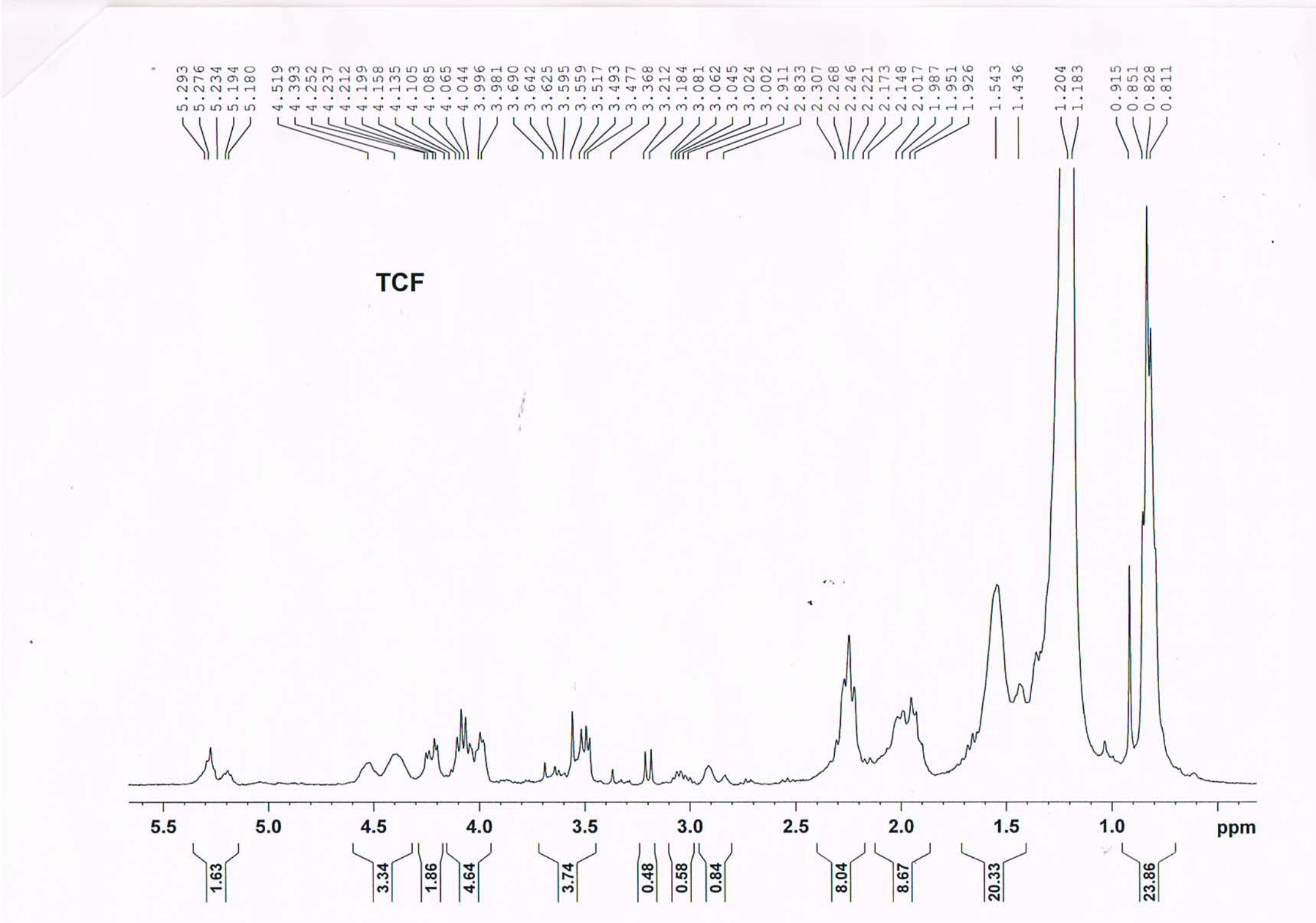
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ACIC
St.Joseph's College (Autonomous)
Trichy-2

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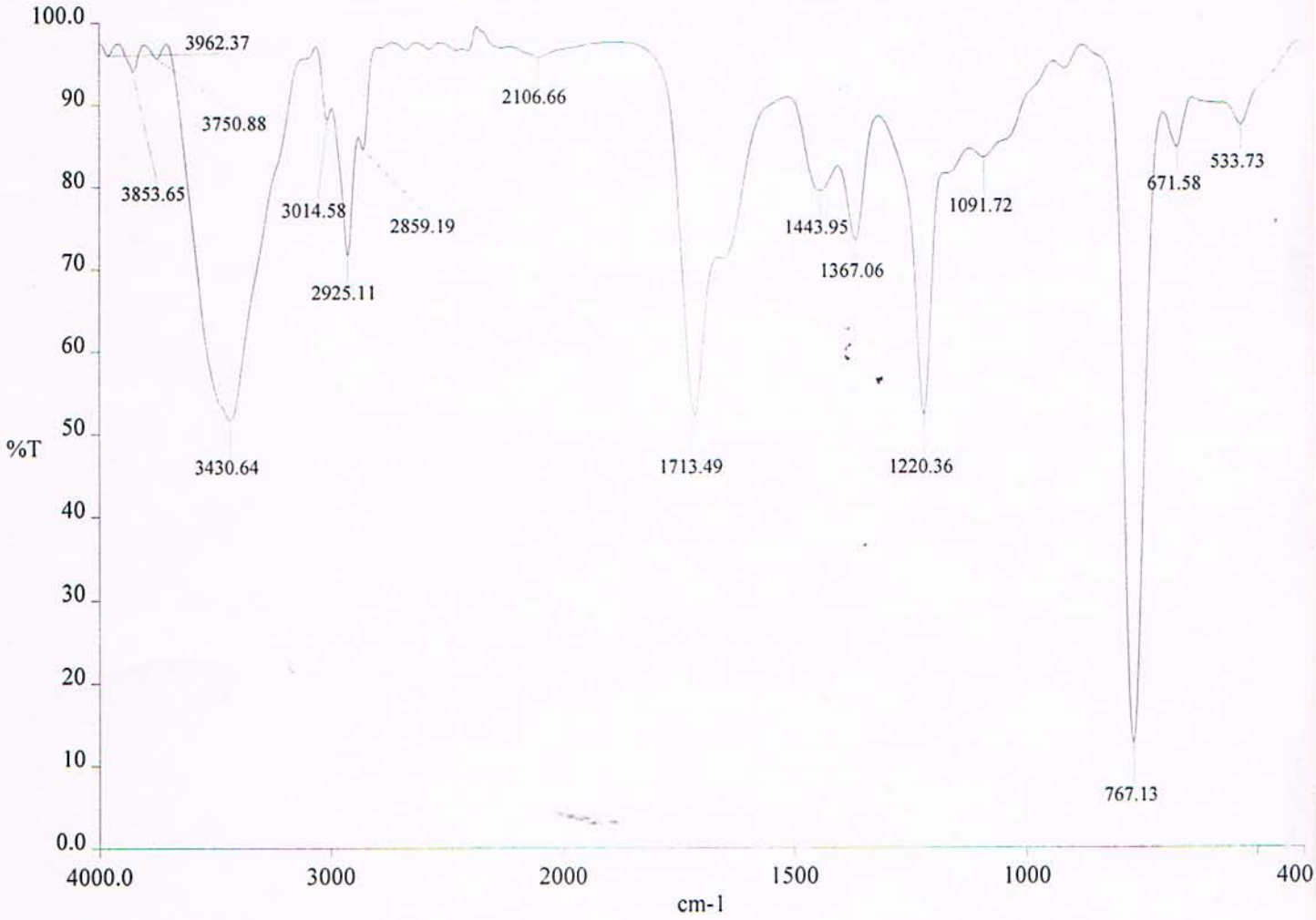

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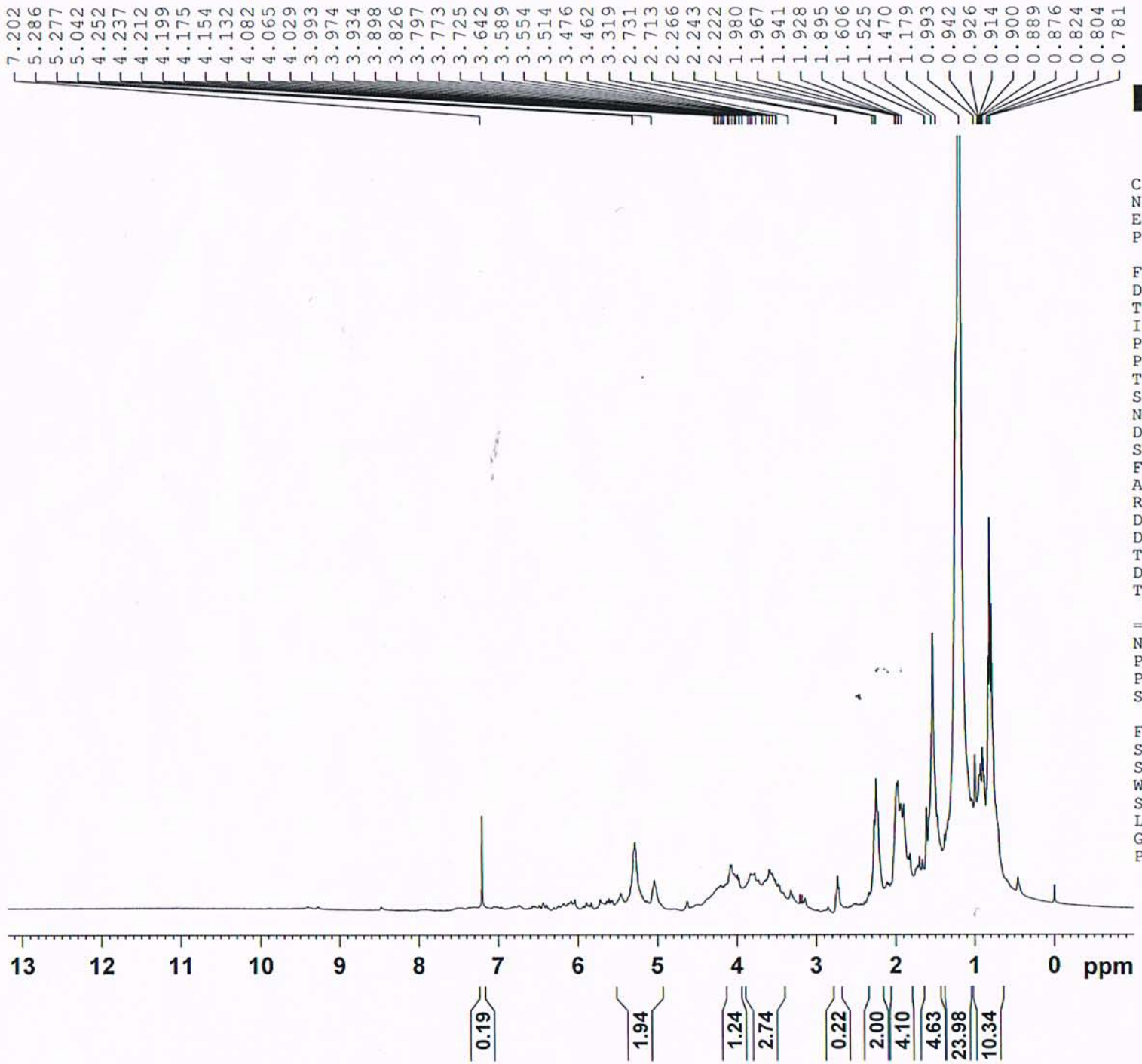

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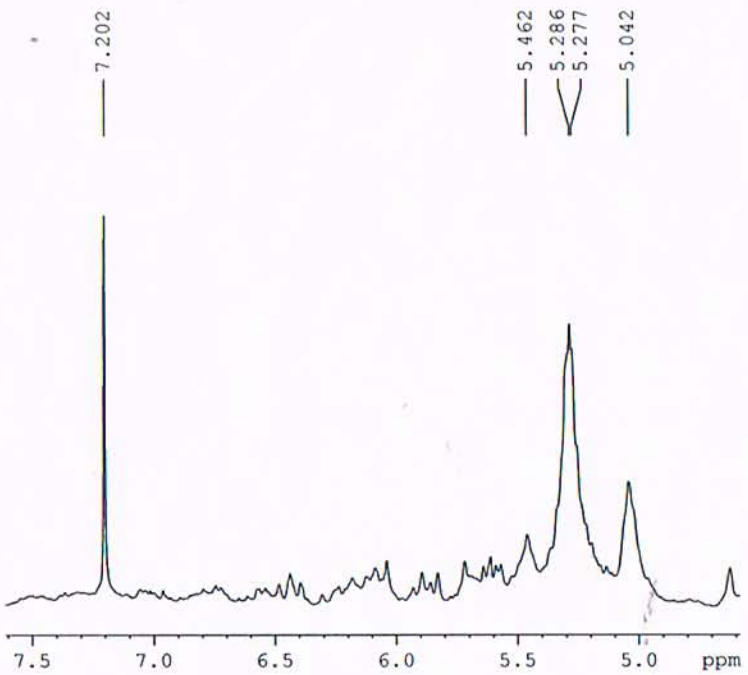
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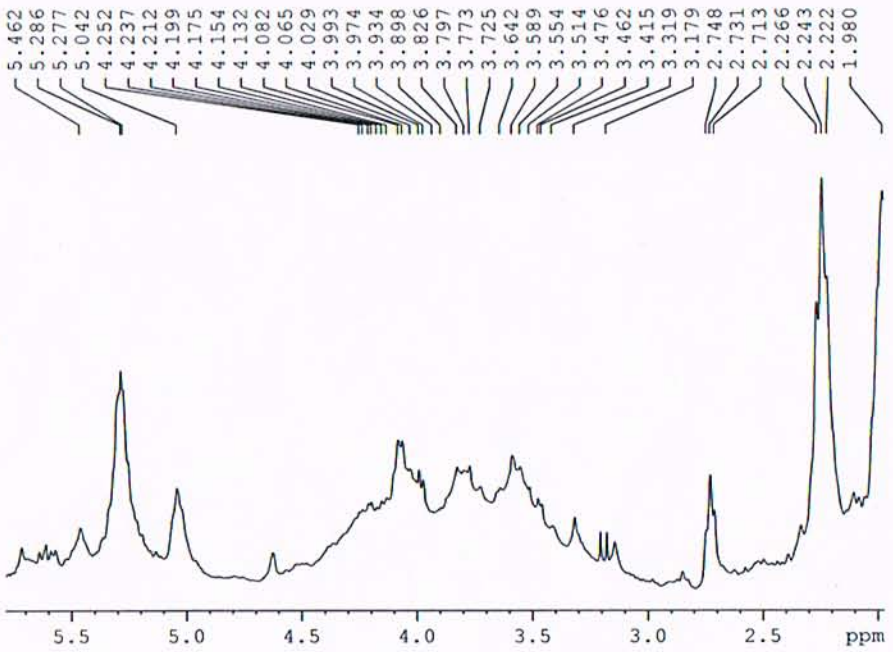
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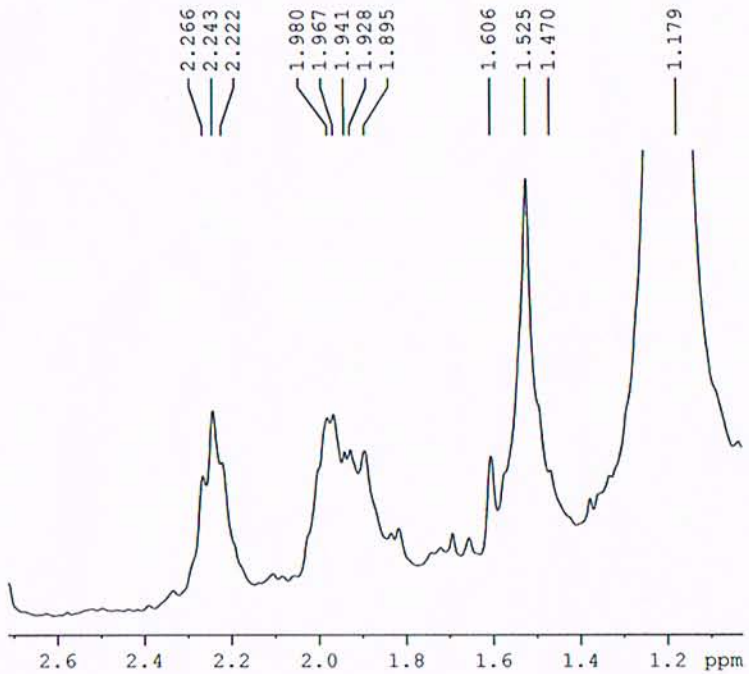
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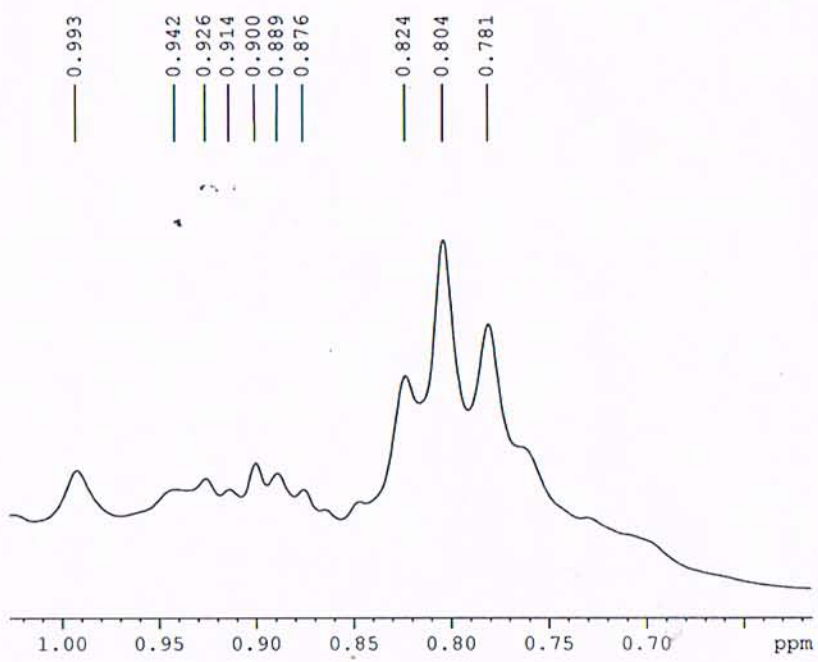
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St.Joseph's College (Autonomous)
Trichy-2

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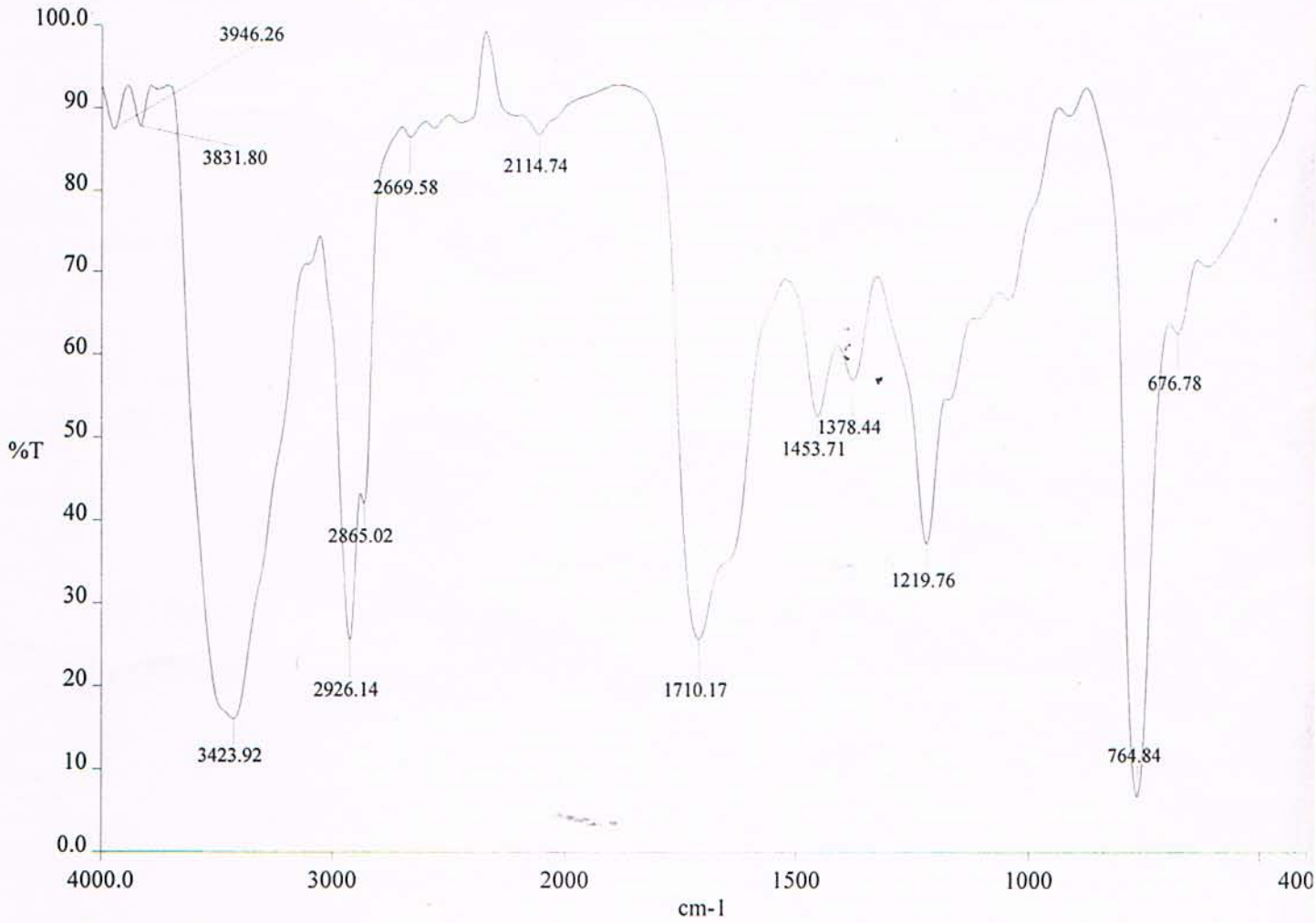

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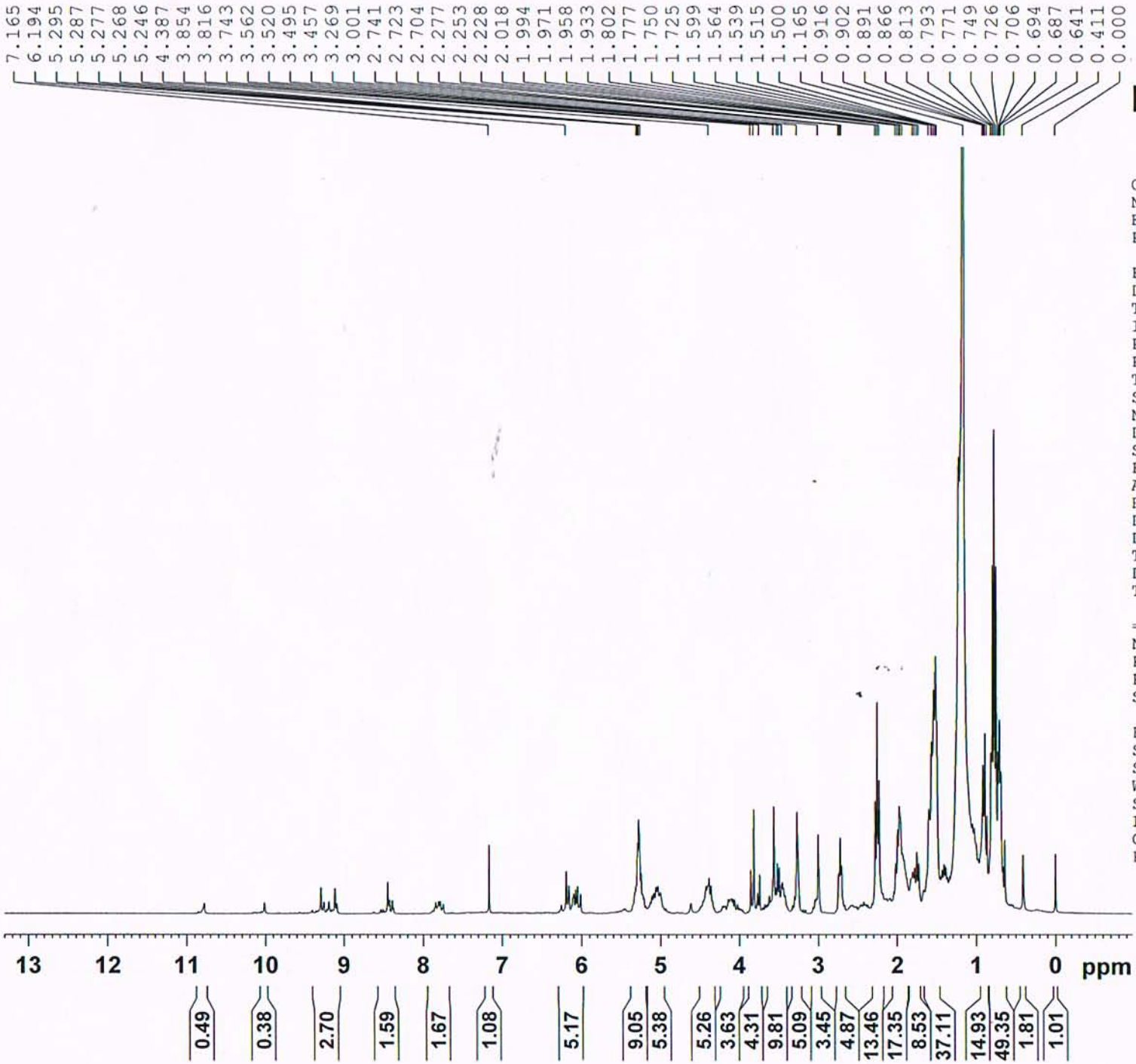

BRUKER

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EXPNO 1
PROCNO 1

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SOLVENT CDCl3
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DS 2
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FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 28.5
DW 80.800 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
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P1 8.60 usec
PL1 -2.00 dB
SFO1 300.1318534 MHz

F2 - Processing parameters
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LB 0.30 Hz
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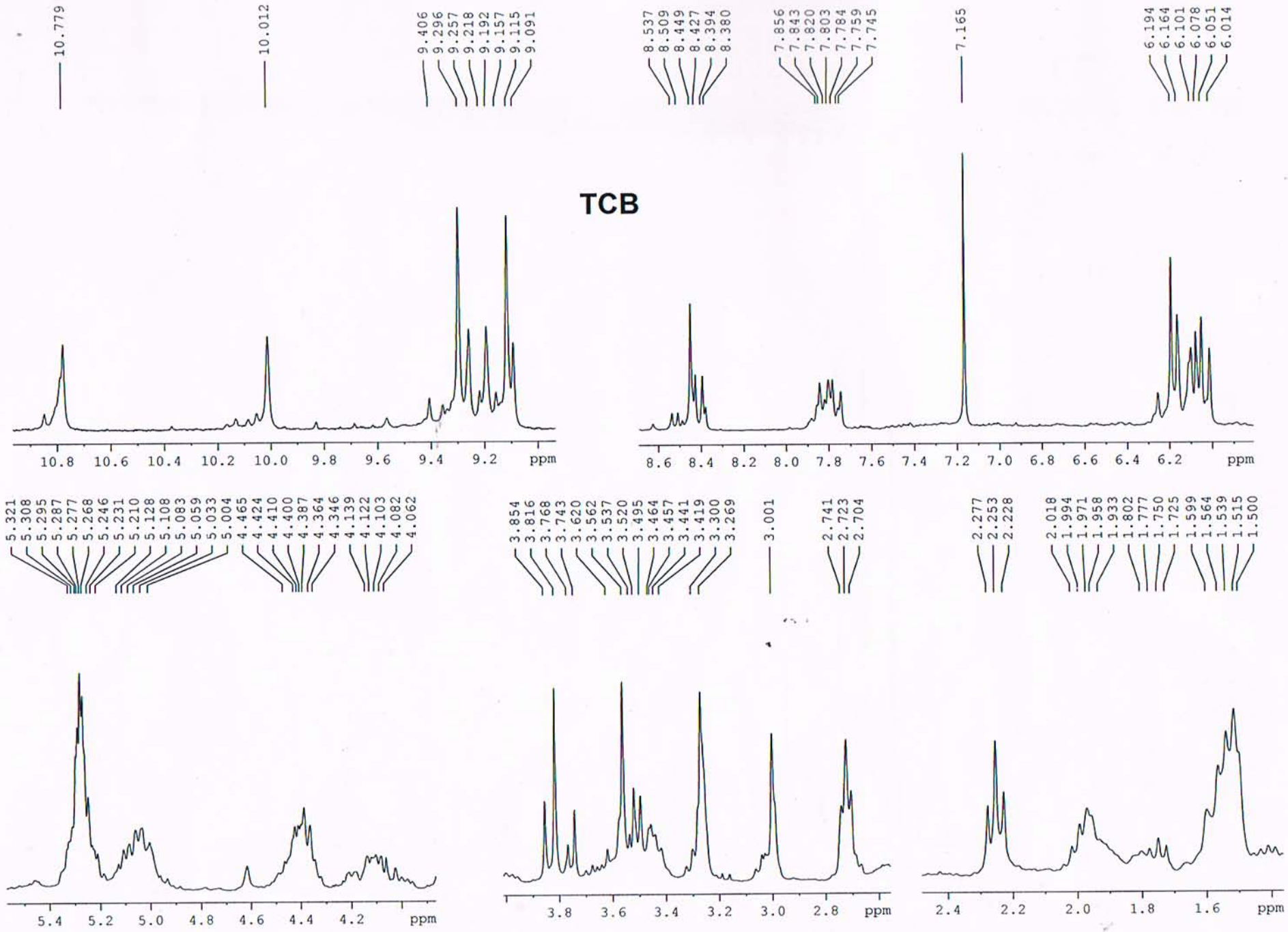
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8.380
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7.745
7.165
6.194
6.164
6.101
6.078
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10.8 10.6 10.4 10.2 10.0 9.8 9.6 9.4 9.2 ppm
8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 ppm
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5.295
5.287
5.277
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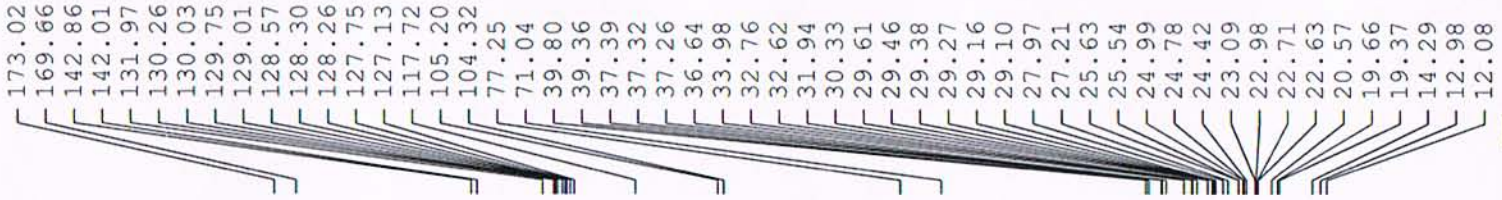

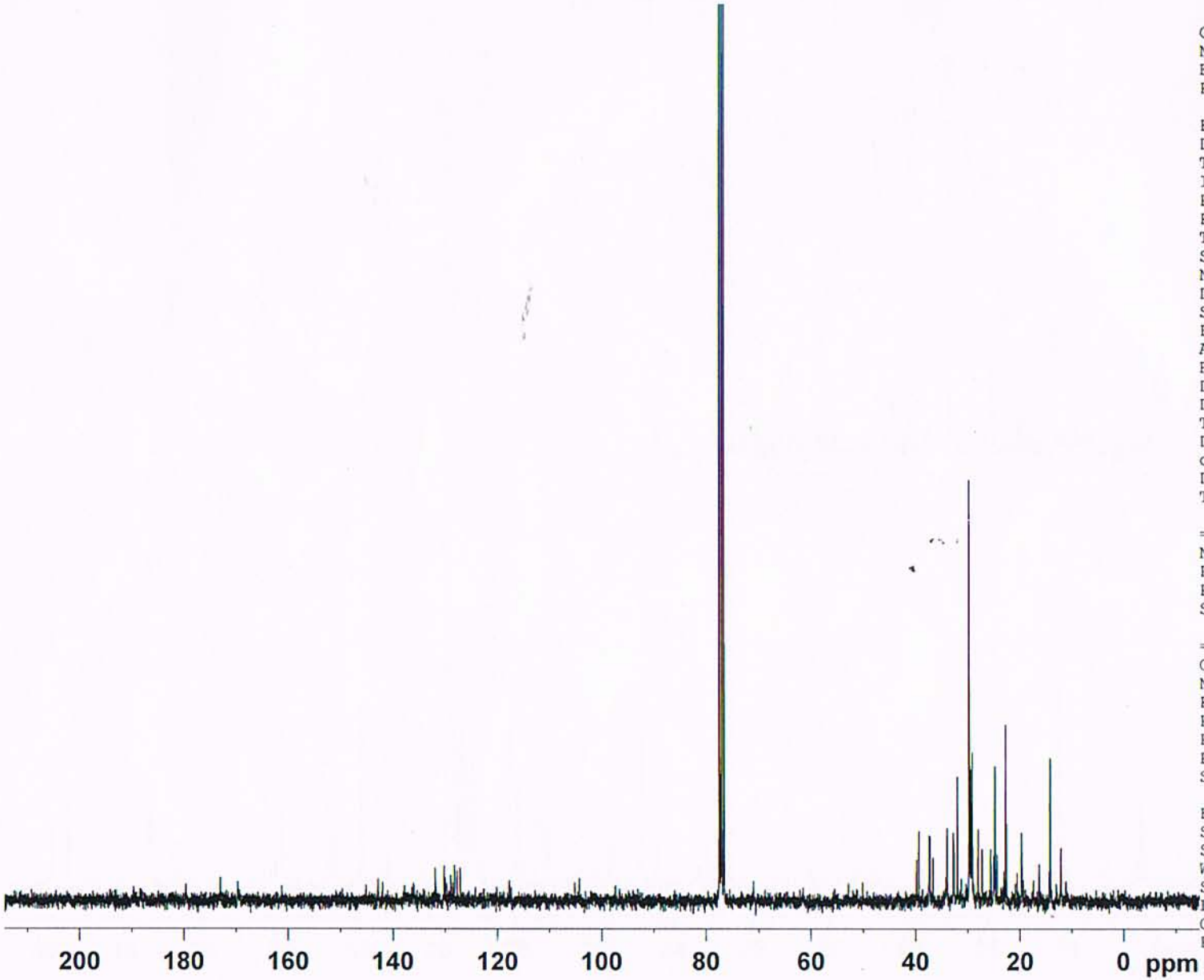

BRUKER

Current Data Parameters

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PROCNO	1

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DS	4
SWH	18028.846 Hz
FIDRES	0.275098 Hz
AQ	1.8175818 sec
RG	512
DW	27.733 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
d11	0.03000000 sec
DELTA	1.89999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	9.50 usec
PL1	0.00 dB
SFO1	75.4752953 MHz

======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-2.00 dB
PL12	17.37 dB
PL13	20.00 dB
SFO2	300.1312005 MHz

F2 - Processing parameters

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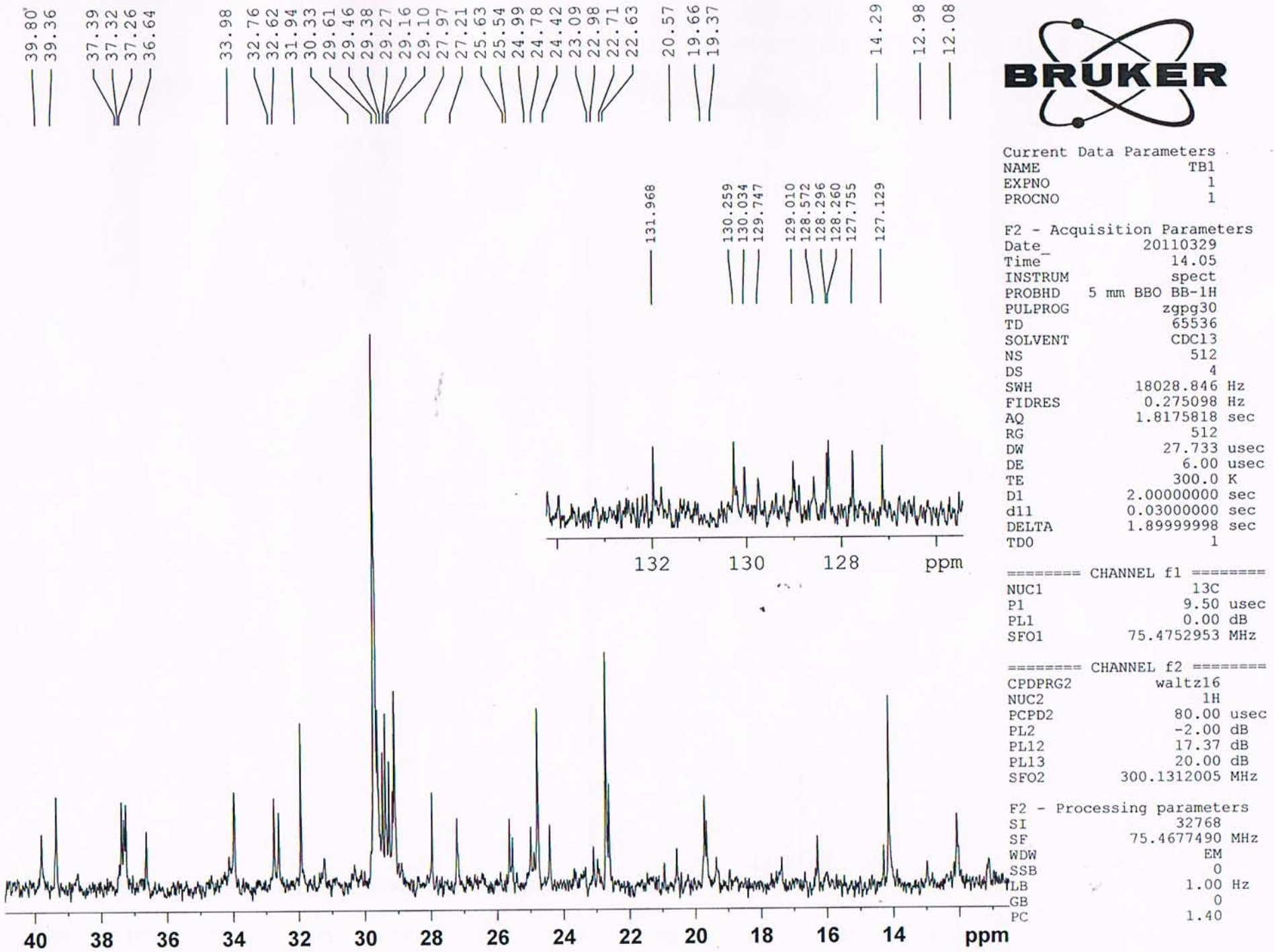

BRUKER

Current Data Parameters

NAME	TB1
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20110329
Time	14.05
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PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	512
DS	4
SWH	18028.846 Hz
FIDRES	0.275098 Hz
AQ	1.8175818 sec
RG	512
DW	27.733 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
d11	0.03000000 sec
DELTA	1.89999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	9.50 usec
PL1	0.00 dB
SFO1	75.4752953 MHz

======== CHANNEL f2 ========

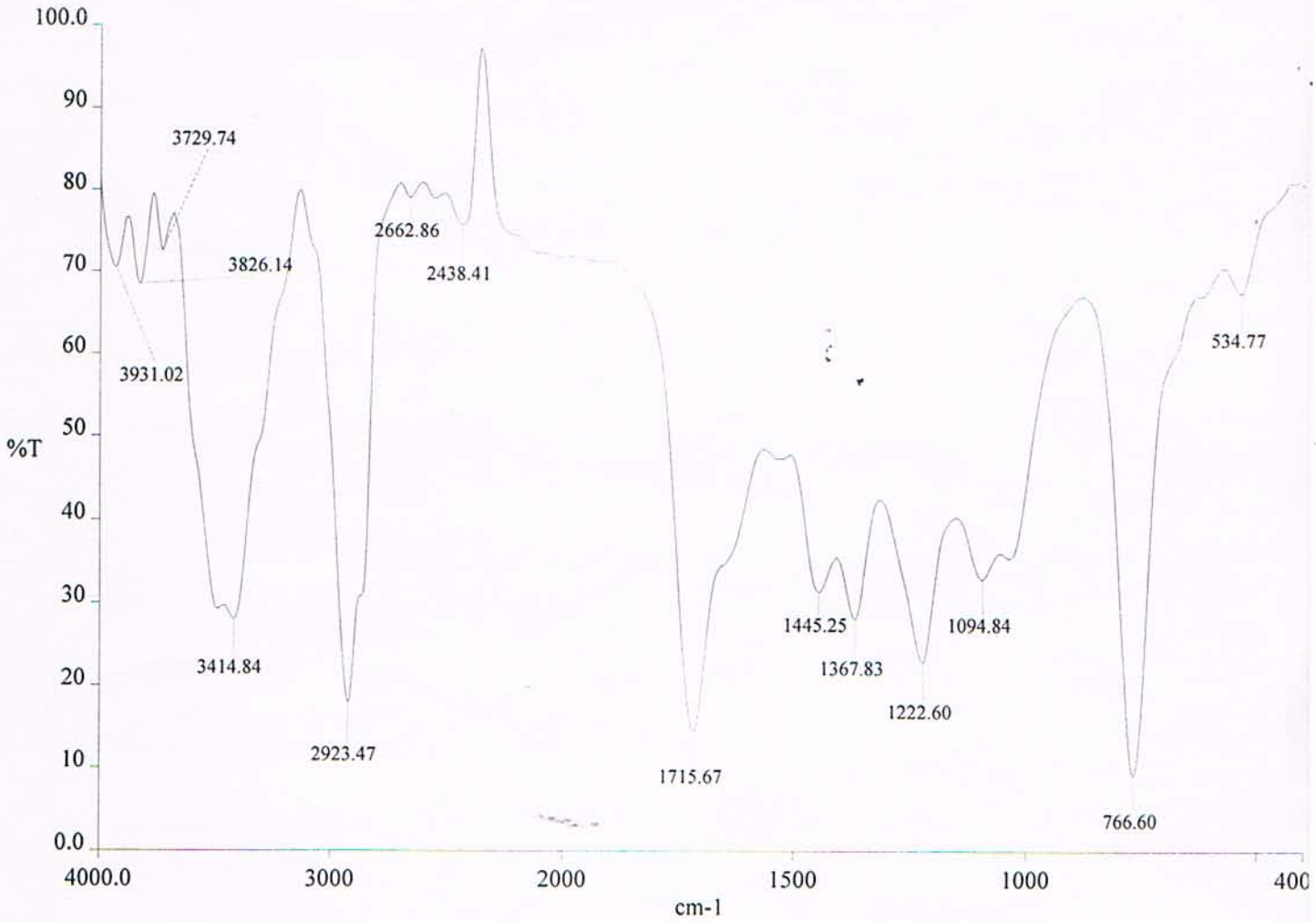
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-2.00 dB
PL12	17.37 dB
PL13	20.00 dB
SFO2	300.1312005 MHz

F2 - Processing parameters

SI	32768
SF	75.4677490 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM
Date: 26-3-2011
Spectrum Name: Sample--C--.sp
Instrument Model: Spectrum RXI



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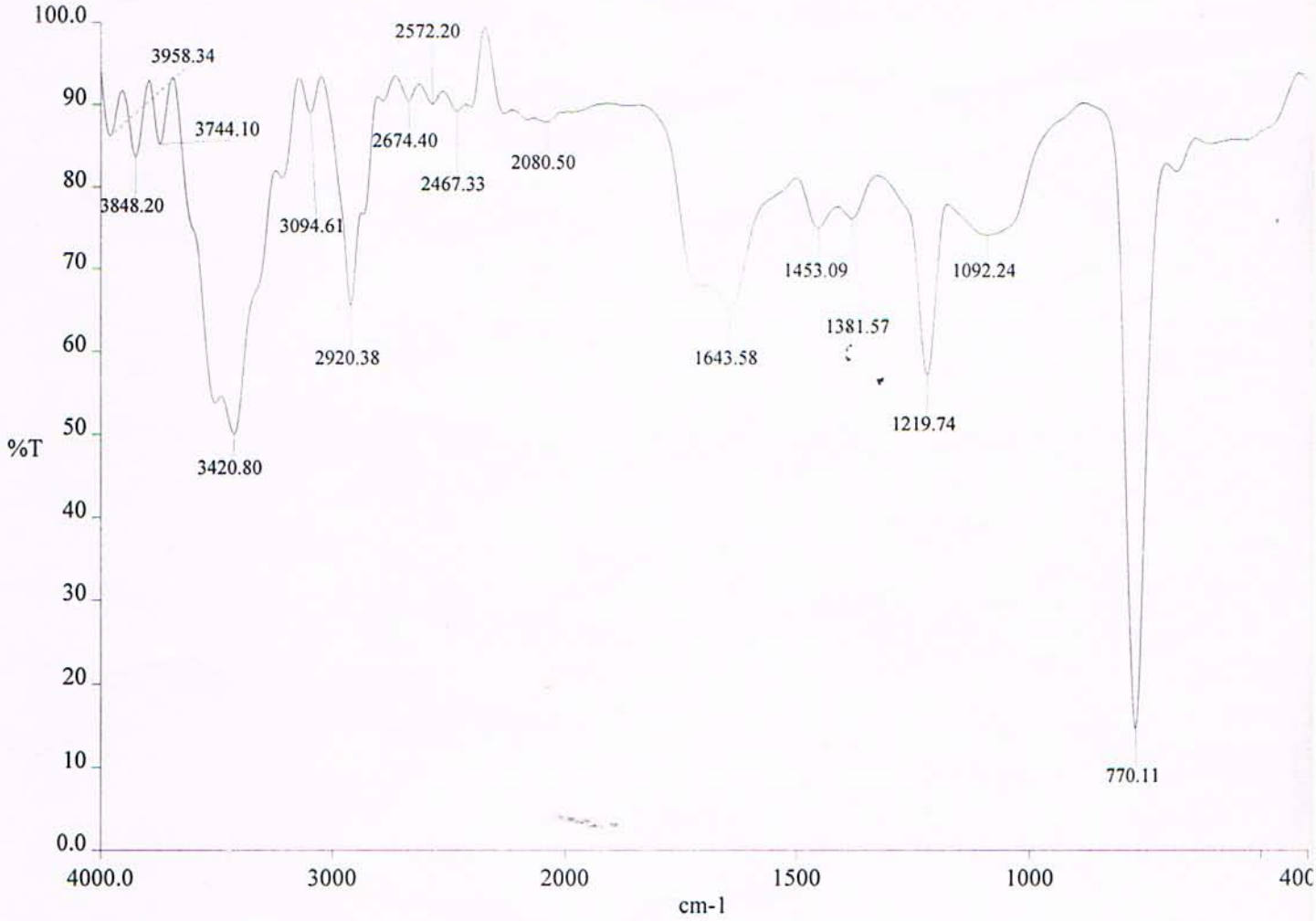
2662.86 79.16 2438.41 75.85 1715.67 14.43 1445.25 31.46 1367.83 28.28
1222.60 22.93 1094.84 32.98 766.60 9.24 534.77 67.56

ACIC
St.Joseph's College (Autonomous)
Trichy-2

FTIR SPECTRUM
Date: 26-3-2011
Spectrum Name: Sample--D--.sp
Instrument Model: Spectrum RXI



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2920.38 65.58 2674.40 90.49 2572.20 90.05 2467.33 89.23 2080.50 87.92
1643.58 65.07 1453.09 74.96 1381.57 76.16 1219.74 57.18 1092.24 74.16
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15/3/11

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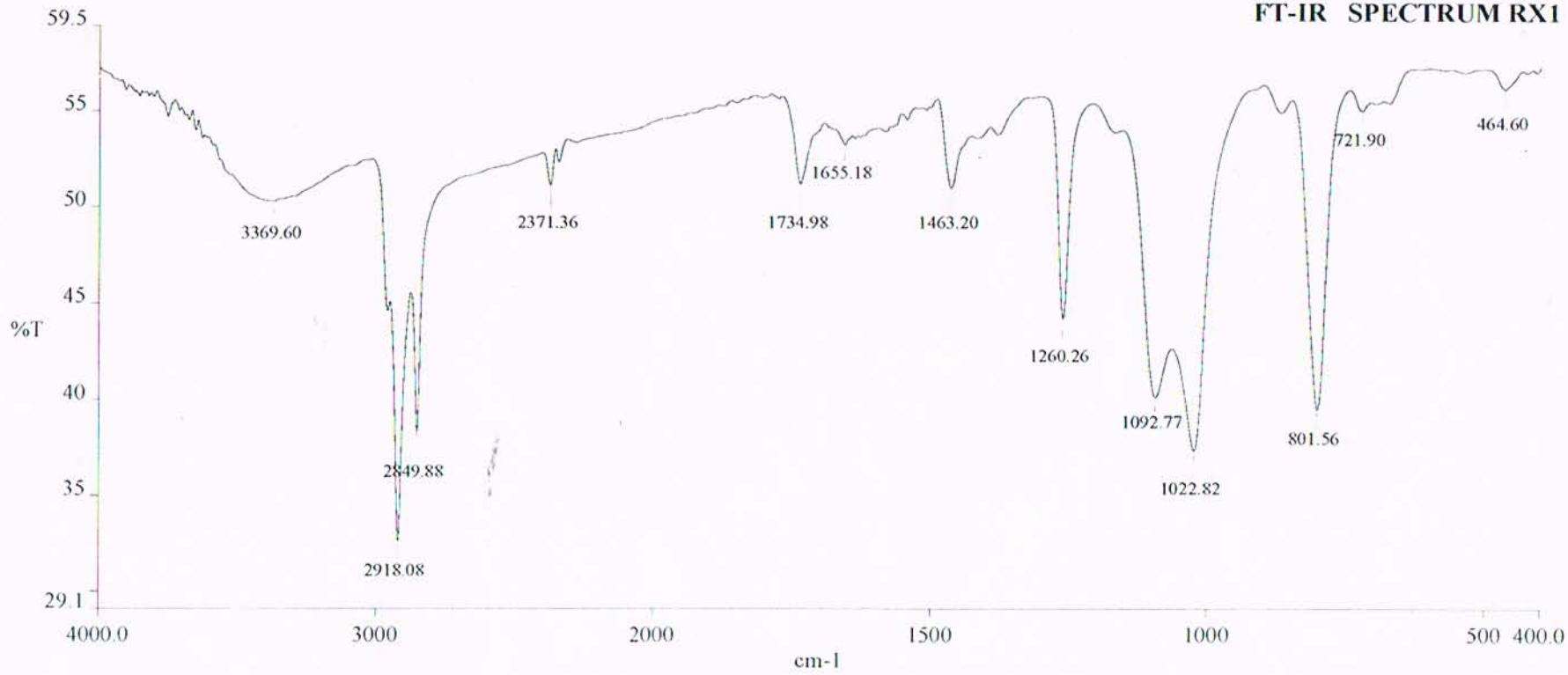
Description: TE

KMCP

FT-IR SPECTRUM RX1



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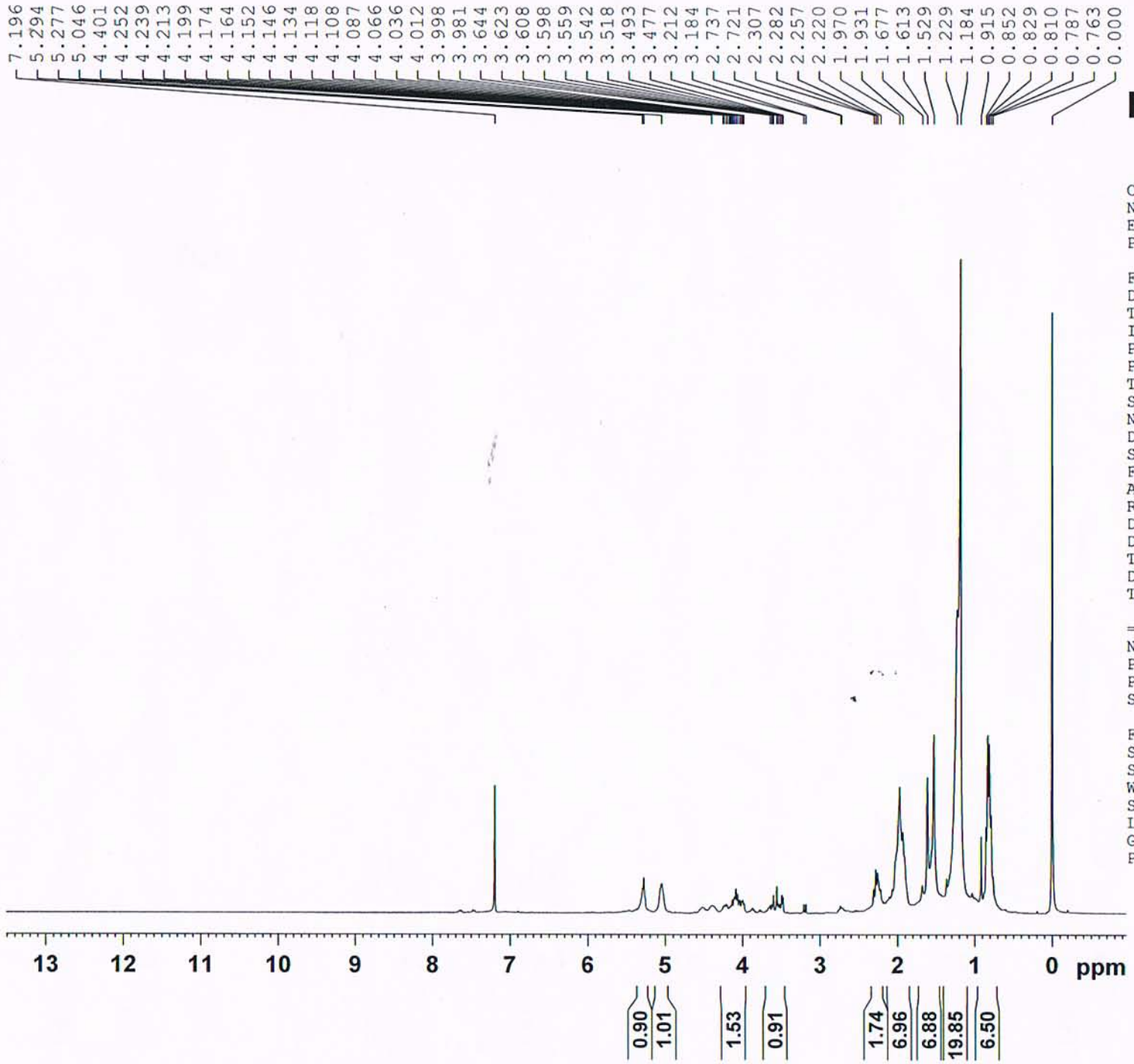

BRUKER

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PROCNO 1

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SOLVENT CDCl3
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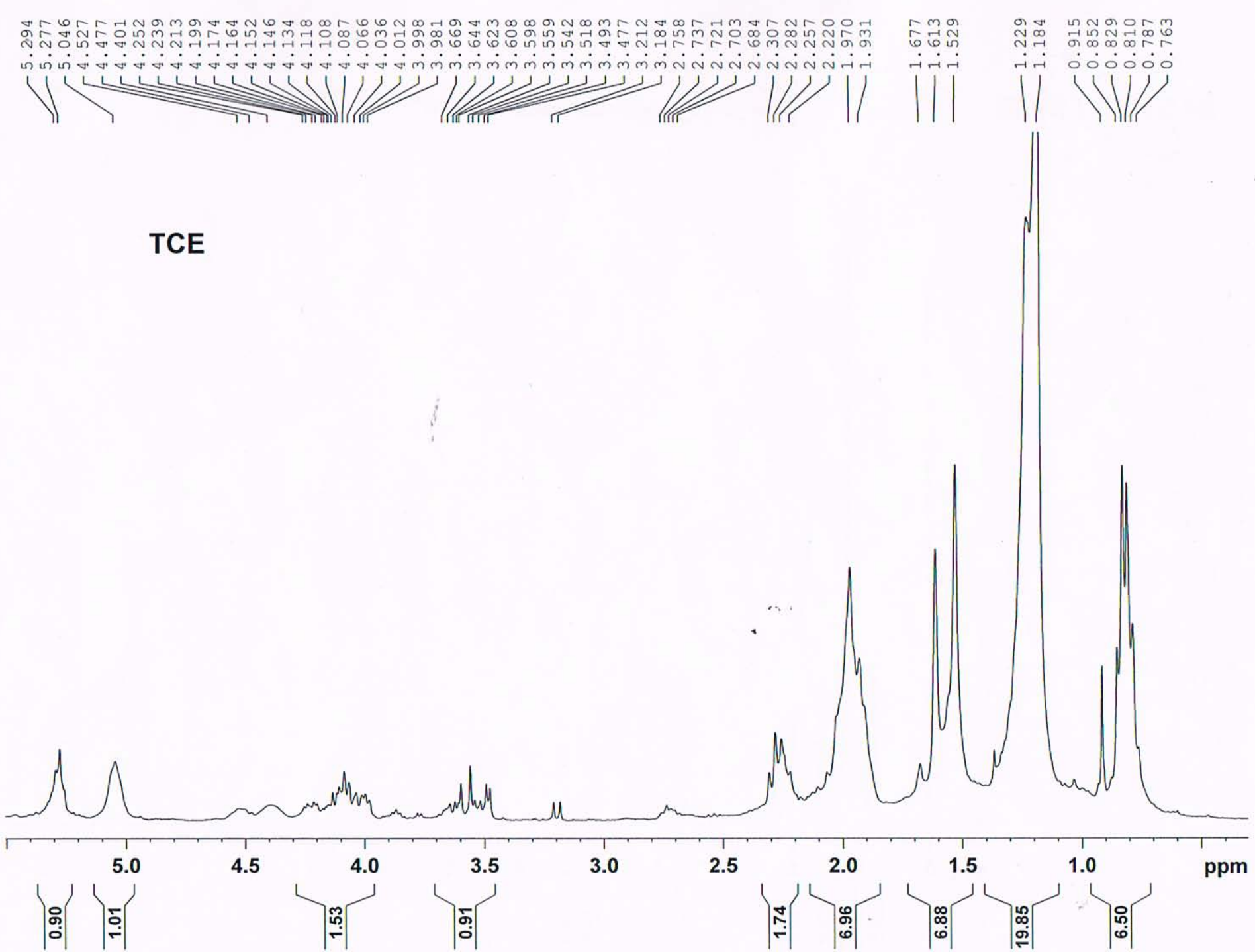
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 80.6
DW 80.800 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 8.60 usec
PL1 -2.00 dB
SFO1 300.1318534 MHz

F2 - Processing parameters
SI 32768
SF 300.1300259 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00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3.542
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TCE
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3.5
3.0
2.5
2.0
1.5
1.0
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19.85
6.50

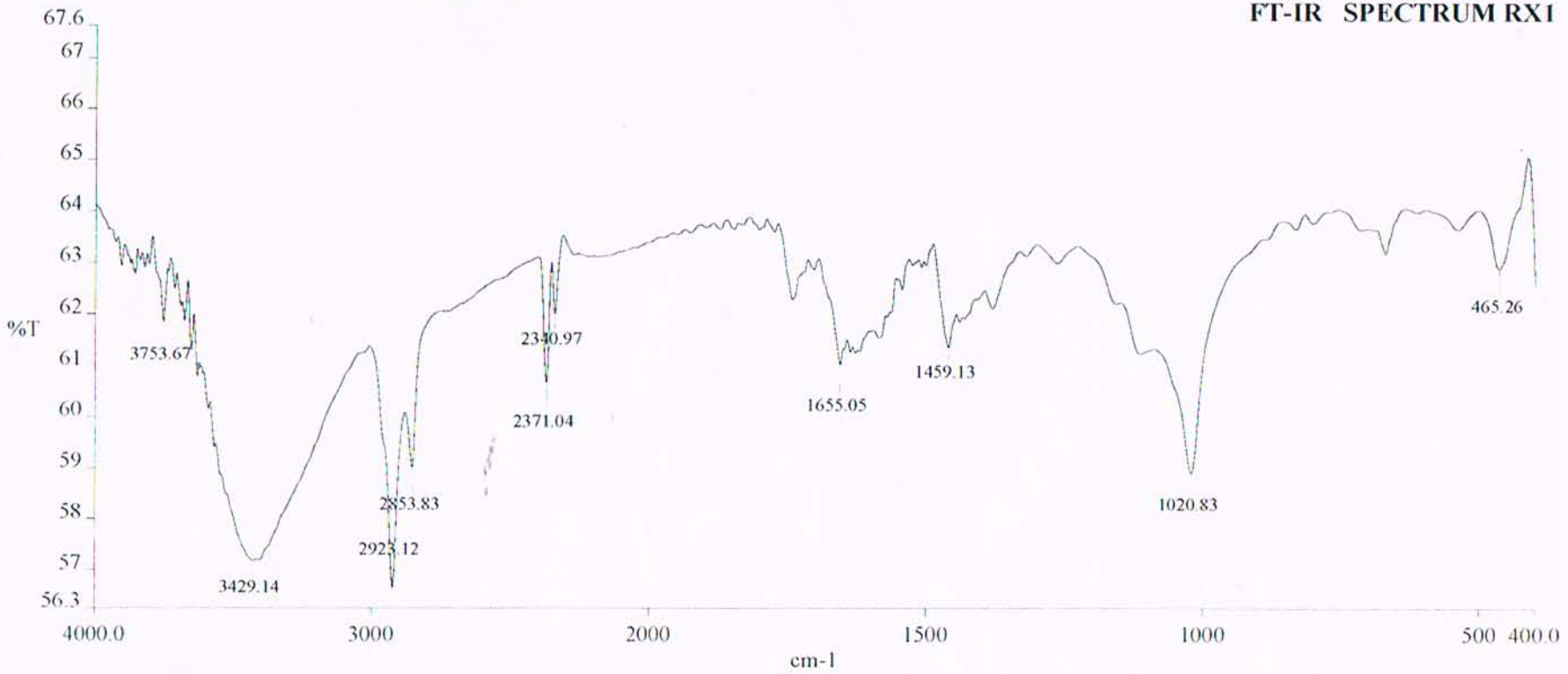
15/3/11

11:30:31

KMCP

Description: TF

FT-IR SPECTRUM RX1



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TF.pk

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TF
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3753.67 61.83 3429.14 57.17 2923.12 56.63 2853.83 59.00 2371.04 60.65
2340.97 62.00 1655.05 61.00 1459.13 61.35 1020.83 58.89 465.26 62.88

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Dr.D.Stephan
Lecturer in Botany

The American College
Madurai

CERTIFICATE

This is to certify that the plant specimen brought to me by Mr.P.NATARAJ, II M.Pharm student of K.M College of Pharmacy, Madurai has been identified as Trichosanthes cucumerina belongs to the family Cucurbitaceae.

SIGNATURE